1974

Host-parasite Relationships Between Trichobilharzia Ocellata And Ducks Previously Exposed To The Same Parasite

James C. Ellis

HOST-PARASITE RELATIONSHIPS BETWEEN *TRICHOBILHARZIA OCELLATA* AND DUCKS PREVIOUSLY EXPOSED TO THE SAME PARASITE

by

James C. Ellis

Department of Zoology

Submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

Faculty of Graduate Studies

The University of Western Ontario

London, Ontario

November, 1973

ABSTRACT

The author undertook to discover how the immune response acquired by ducks after a primary exposure to Trichobilharzia ocellata affects challenging parasites, and to determine if this immunity is exercised through cells, antibodies, or a combination of the two.

Migration of worms in immune birds, studied in teased, squashed, and sectioned tissue, revealed that parasites were retained in the skin, mainly in the stratum corneum, up to seven days. Many reached the lungs but adhered to bronchial walls in mucoid material. Worms from first challenge exposures appeared in the liver in sinusoids surrounded by lymphocytes, but few specimens were found in the liver in subsequent challenges. Apparently no worms reached the host's intestinal veins or tissues.

Worms which survived seven days in immune hosts reached about 300 mm in length, approximately 10 - 15 % of the normal value. Those from 16 of 19 birds retained cercarial features such as tegumental spines, eye spots, and head organs, although some parasites in the remaining three birds showed normal or near normal maturation.

The ultrastructure of T. ocellata's tegument and gut not previously recorded, proved to be similar to that described for other species. Examination of worms recovered from immune hosts showed no lesion or conspicuous architectural derangement. Peroxidase-labelled

rabbit anti-duck gamma globulin indicated that antibody was adhering to the tegument, and benzpyrene fluorochrome technique showed that parasite membranes were indeed injured by some mechanism within the immune host. Attempts to suppress the immune response by total body gamma irradiation from a cobalt 60 source failed in that all birds tested developed immunity to reinfection by I. ocellata. Finally, all efforts to transfer immunity passively failed when spleen or diverticulum cells, moderate amounts of serum, or combination of cells and serum was used. However, when 28 - 60 ml of hyperimmune serum were injected over 2 - 5 days the worms removed were stunted in comparison to those from control birds, and ducks injected with 2 ml hyperimmune serum every 8 hours for 12 days either failed to develop a patent infection when challenged or tardily showed a low grade parasitemia.

ACKNOWLEDGEMENTS

The list of people to whom gratitude is due is very long indeed. To the Department of Zoology and its chairman, Dr. Michael Locke, I am indebted for the use of excellent facilities both in our own laboratory and in the E M unit. With regard to the electron microscopic part of the work, Mr. Artie Lyness and Miss Terry Kenny were of great assistance and took a real interest in the results. I should also like to thank Dr. R. R. Shivers for his advice in interpreting the electron micrographs.

Dr. N. R. Sinclair of the Department of Bacteriology and Immunology was of much assistance in discussing the thesis and planning subsequent strategy for which I am sincerely grateful. Thanks are also due Dr. W. R. Inch of the staff of Victoria Hospital for introducing me to the irradiation procedures.

To Mrs. Merilyn McCann who provided much needed technical help as well as many hours of interesting discussion, and to fellow student, Mrs. Pat Smith, I express my gratitude.

For permission to pursue this study and encouragement in many ways, I am indebted to the Salvation Army and especially fellow officers, Brigadiers Cyril Boyden and Edward Read.

My chief advisor, Dr. Kenneth Bourns, made parasitology a

fascinating area of study for me as an undergraduate. I will always be grateful for his friendship, for the knowledge shared over five years, and for his faith in me as a person.

To my patient and persistent wife who has been a constant source of encouragement and who is responsible for the line drawing of the adult schistosome tegument as well as the histograms and graphs included in this thesis, I also say thankyou.

This study was made possible by the National Research Council of Canada in the form of a personal scholarship to the author and in grants in aid to Dr. T. K. S. Bourns.

It remains to express my gratitude to my wife's sister, Mrs. Ruth Kew, for typing the manuscript under rather unusual conditions. Thank you for a careful job, well done.

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processed (Rau, 1969) to establish the pattern of numbers of viable miracidia produced by each bird.

After the primary infections had terminated (egg passage 9 after day 40) each bird was challenged and the numbers of miracidia determined as before.

IX ADOPTIVE TRANSFER AND PASSIVE TRANSFER:

White pekin ducklings were injected intraperitoneally with serum and/or lymphoid cells from hyperimmune ducks (birds exposed 3 or more times to cercariae at intervals of not less than 3 weeks). Serum for each experiment was pooled from 3 or more birds, sterilized by Millipore filtration, and stored at 4° C. Cells were obtained when aseptically-removed spleens and Meckel's diverticulum tissues were forced through nylon mesh into sterile balanced buffered saline solution (BBSS) (Appendix IIA) at 4° C (hunter et al., 1967), centrifuged 5 - 7 minutes at 1000 rpm, and resuspended in cold BBSS. Cell counts were made on an improved Neubauer haemocytometer and cell viability was determined by the eosin exclusion test (Banks and Wallace, 1958).

Unless otherwise noted, each hyperimmune-serum-transfer experiment included normal serum and untreated controls. Experiments with spleen cells were usually performed in duplicate, two birds each receiving half the spleen cells from an hyperimmune donor, two each receiving half of the cells from a normal donor, and two remaining untreated. The same system was used with diverticulum cells but the entire harvest from one donor was injected into one recipient.

At prescribed times after the administration of serum

with S. mansoni (Newsome, 1956) most closely mimics the parasitized human. By contrast, the rhesus monkey, infected with S. mansoni or S. japonicum, has been shown to develop significant protective immunity to homologous challenge (Vogel and Minning, 1953; Naimark et al., 1960; Liu and Bang, 1950, Meleney and Moore, 1954, Sadun, 1963) even after a single exposure to small numbers of cercariae (Smithers and Terry, 1965). For obvious reasons workers the world over continue to study this host-parasite model in the hope of discovering the mechanism(s) by which protective immunity is developed.

In this laboratory the discovery that a related parasite, Trichobilharzia ocellata, induces a similar type of immunity in its duck hosts, led to the initiation of a series of investigations into the biology of this host-parasite model.

Studies on migration, growth, and maturation in fully susceptible birds (Ellis, 1968, Bourns et al., 1973) revealed that sexual maturity and adult size are achieved in 7 days and that egg laying begins on day 9 with peak numbers of eggs appearing in the host's gut wall between days 14 and 16. Schistosomula appear in lung and liver between days 1 and 9 and then migrate to the host's intestinal veins where they may be found between days 9 and 21. Most adults apparently perish after 3 weeks in the host but a few may be found in the liver after more than 12 months.

Rau (1969 and in prep.) exposed black ducks (Anas rubripes) to T. ocellata cercariae and to implanted adults and studied the course of worm egg production by recovering hatched miracidia from the daily fecal output of each host. He found that egg passage

patterns varied, being especially erratic in very young birds and after implantation of adults. In general, however, passage of viable eggs began on day 13 or 14, reached peak numbers 5 or 6 days later, and declined to zero by day 40. When challenged up to 16 months later, birds exhibited significant to total resistance as measured by passage of viable eggs, regardless of the nature of the primary infection.

These early indications led to the consideration that the T. ocellata - duck model may be a near-duplicate of the S. mansoni-rhesus monkey model, even to the point of having adults of the original infection survive the effects of the immune response which they themselves provoked, a phenomenon which Smithers and Terry (1969a) called concomitant immunity. If this possibility is born out in fact, the bird-schistosome system may prove to be useful, especially in view of the rapidity of life cycle events, (7 days to sexual maturity vs. 35 days for S. mansoni (Clegg, 1965), cost, hazard, and the humanitarian principle of using as experimental animals, the least sentient which may be expected to yield needed information. Accordingly, this study was undertaken to gather information about the nature of the relationship between T. ocellata and immune ducks. First, the details of migration, growth, and maturation in previously-sensitized birds were studied, so that these could be compared with the corresponding events in fully susceptible hosts (baseline information relating to primary infections was available as a result of the author's earlier studies but was expanded in the present work). Second, histological and cytological examinations were made of

parasites from susceptible and immune ducks in the hope of learning something about the nature of the immune lesions produced in the latter. And finally, attempts were made to transfer immunity passively in the hope of establishing whether cells, antibodies, or both, are responsible for the protective immunity in question.

MATERIALS AND METHODS

I ANIMALS:

Trichobilharzia ocellata, originating near Peterborough, Ontario, has been maintained in this laboratory for more than 10 years by successive passage through lab-reared snails (*Lymnaea stagnalis*) and several species of ducks, wild species being purchased from Kortwright Waterfowl Research Station, Guelph, Ontario, and domestics from Webfoot Hatchery, Elora, Ontario, or King Cole Duck Farms, Aurora, Ontario. White Pekins (*Anas platyrhynchos*) were used for all transfer experiments and both White Pekins and Black Ducks (*Anas rubripes*) were used for other parts of this work.

II EXPOSURE TO PARASITES:

Usually, birds were exposed individually to *T. ocellata* when one foot was held for 90 minutes in a jar of water containing thousands of freshly-shed cercariae from five or more snails. No effort was made to count cercariae. When attempts to transfer immunity passively were being challenged, however, sets of experimental and control birds stood together in single large dishes of cercarial suspension.

III RECOVERY OF PARASITES: (for study of migration and development)

Birds were killed by exsanguination after cardiac puncture and then parasites were sought in squashed or teased tissue.

Approximately 1/8 of the liver, one entire lung, 1/3 of one kidney, and 1/2 of the exposed web were examined. Many of the delicate worms were broken by these procedures making it difficult to determine accurately the absolute numbers of specimens present. Estimates of relative abundance, on the other hand, were confirmed by study of sections and appear to be reliable. When growth and maturation were being considered, worms were fixed in Gilson's fluid, stained in Mayer's Carmalum, dehydrated, and mounted whole in Permount (Fisher Scientific). Measurements were made either directly with an ocular micrometer or indirectly by map tracer measurement of lines drawn through the median aspects of projected photographic images.

IV DETERMINATION OF SPECIFIC LOCATION IN HOST:

With a view to determining the exact location of parasites within the host, samples of liver, lung, kidney, web, and gut were fixed in Davison's fluid (Appendix I). Histological sections (7 um, paraffin) were stained with Ehrlich's alum haematoxylin and counterstained with Bowie's eosin and Orange G. A block of tissue at least 1 cm^3 was examined in each case except the gut where 1/2 of a 2.5 cm. length containing either Meckel's diverticulum or the third lymphoid ring (Ellis, 1968; Leibovitz and Uwang, 1968) was examined in tangential sections.

V ULTRASTRUCTURE OF SCHISTOSOMULA FROM PRIMARY AND CHALLENGE INFECTIONS:

Worms recovered from lungs or liver at days 2, 3, 4, 8, 9 and 10 of initial infections and days 3, 5, 6 and 9 of challenge infections were fixed in cold 3 % glutaraldehyde in 0.12 M (pH 7.3)

phosphate buffer (Millonig, 1961) for 4 h. After being washed in buffer (several changes) worms were post-fixed in cold, buffered osmium tetroxide (2 %) for 1 h, washed briefly in water, dehydrated in ethanol, and embedded in Epon or Araldite via propylene oxide (Pease, 1964). Ultra-thin sections were made on an LKB or Reichert ultramicrotome and stained by inverting the grids on successive drops of lead citrate (Reynolds, 1963) and saturated uranyl acetate (5 minutes each). A Philips EM-100 or 201 was used to make micrographs at original diameters of 650 to 19000X. With the exception of day 9 challenge when only one worm was recovered, 3 to 6 specimens were examined in each case, sections being made at several points along the lengths of the worms.

VI BENZPYRENE TECHNIQUE - THE INTEGRITY OF PLASMA MEMBRANES:

A 0.75 mg. 3:4 - Benzpyrene/100 ml 1/2 saturated caffeine solution was prepared according to the method of Bitensky (1963).

Worms recovered (from lungs) of primary or challenge infections in ducks were pipetted into a well-slice in 1 ml. of 0.85 % NaCl. After 0.6 mls benzpyrene solution was added, the slide was rotated for 5 - 7 minutes (clinical rotator, Fisher) at 90 rpm, the worms washed in fresh saline, and then examined under uv illumination with a Zeiss fluorescent microscope. The results were recorded on Kodak High Speed Ektachrome film.

VII PEROXIDASE TECHNIQUE - LOCATION OF ANTIBODIES ON SCHISTOSOMULA:

Protocols for the coupling of peroxidase to gamma globulin and details of this technique have been outlined by Nakane and Pierce (1966) and Leduc et al. (1969). The exact procedure

followed in this work is given in Appendix II (Jones, personal communication).

Worms were removed from lungs of a normal and a hyperimmune duck at day 3 and fixed for 4 h (3 % glutaraldehyde) at 4° C. They were then washed for several hours in PBS at 4° C (several changes) before being reacted with rabbit anti-duck peroxidase-labelled gamma globulin for 12 h at 4° C.

The worms were then washed in PBS at 4° C overnight, fixed in 5 % glutaraldehyde (pH 7.2-7.3) for 1 h and washed in cold (4° C) PBS overnight (several changes) before being stained for peroxidase. The Graham and Karnovsky method (1966) was used to identify peroxidase and is included in Appendix II. Blocks were sectioned and examined in the EM without further staining.

VIII IMMUNOSUPPRESSION:

i) When 2 or 3 days old, 12 ducklings were exposed to total body gamma irradiation from a cobalt 60 source (Victoria Hospital, London, Ontario). Two birds were exposed to each of 400, 500, 600, 700, 800 and 900 rads, all administered at 54 - 58 rads/min. Four non-irradiated ducklings served as controls. Leucocytes were counted 6 or 7 days after irradiation and again 4 - 16 days later. The direct method employing Brilliant Cresyl Blue to make thrombocytes distinguishable from leucocytes (Lucas and Jamroz, 1961 p. 233) was used, optimal lighting of the Neubauer chamber being provided by a phase contrast condenser combined with a normal objective.

Nine ducklings were exposed to cercariae 9 - 18 days after irradiation. Their feces were collected in 0.85 % NaCl and

processed (Rau, 1969) to establish the pattern of numbers of viable miracidia produced by each bird.

After the primary infections had terminated (egg passage 0 after day 40) each bird was challenged and the numbers of miracidia determined as before.

IX ADOPTIVE TRANSFER AND PASSIVE TRANSFER:

White pekin ducklings were injected intraperitoneally with serum and/or lymphoid cells from hyperimmune ducks (birds exposed 3 or more times to cercariae at intervals of not less than 3 weeks). Serum for each experiment was pooled from 3 or more birds, sterilized by Millipore filtration, and stored at 4° C. Cells were obtained when aseptically-removed spleens and Meckel's diverticulum tissues were forced through nylon mesh into sterile balanced buffered saline solution (BBSS) (Appendix IIA) at 4° C (Hunter et al., 1967), centrifuged 5 - 7 minutes at 1000 rpm, and resuspended in cold BBSS. Cell counts were made on an improved Neubauer haemocytometer and cell viability was determined by the eosin exclusion test (Hanks and Wallace, 1958).

Unless otherwise noted, each hyperimmune-serum-transfer experiment included normal serum and untreated controls. Experiments with spleen cells were usually performed in duplicate, two birds each receiving half the spleen cells from an hyperimmune donor, two each receiving half of the cells from a normal donor, and two remaining untreated. The same system was used with diverticulum cells but the entire harvest from one donor was injected into one recipient.

At prescribed times after the administration of serum

and/or cells, the birds were exposed to cercariae by foot immersion. One group which received serum was kept for 20 days, then the feces of each duckling were processed (Rau, 1969) and the numbers of miracidia counted. All other birds were killed 3 - 5 days after cercarial challenge, and worms recovered from teased lung were fixed in Gilson's fluid and measured as outlined above.

Massive amounts of serum were used in 2 further experiments on passive transfer. In the first of these intraperitoneal injections began when the 12 birds were 1 day old and continued at 12 hr intervals. The objective being to introduce as much serum as the peritoneal cavity would accept, the amount increased as the birds grew. In each case, however, controls received the same volume of normal serum or saline as the test birds received of hyperimmune serum. Sets of three ducklings (1 test and 2 controls) were challenged by cercariae at 2, 3, 4, and 5 days of age. Four days after challenge the birds were killed and all schistosomula found in the lungs were fixed and measured. Total volumes of fluid injected were 28.5, 37.8, 44.8, and 60.0 ml. in the 4 sets of birds.

In the second experiment 15 ducklings were exposed to cercariae when 1 day old. Beginning at the same time and continuing every 8 hr. for 12 days, 5 birds were injected i.p. with 2 ml. hyperimmune serum (3 duck pool), 5 with normal adult serum, and 5 with sterile saline. Feces were collected between days 8 and 30 and numbers of viable miracidia determined as outlined previously.

RESULTS

I MIGRATION:

In contrast to the rapid passage through duck skin which was exhibited by T. ocellata in initial infections (Ellis, 1968), parasites were seen for several days in the skin tissue of birds which had experienced one or more previous exposures. Schistosomula were found in skin for 3 days after the first challenge exposure (fig. 1) and sections revealed that few of these were located beneath the stratum corneum. All 34 in a bird on day 1 were found in the stratum corneum while in a second duck, besides 5 found in the stratum corneum, 1 was discovered in the stratum germinativum and 1 in the dermis. On day 2, 20 were found in the stratum corneum while the stratum germinativum, dermis, and hypodermis contained 1 each. None was detected below the stratum corneum on day 3 (see fig. 2). An intense inflammatory response was detectable within the skin after the disappearance of the worms (fig. 3).

After second challenge exposure, schistosomula were found in skin as late as day 6 (fig. 4). The following distribution was revealed by sections at 10 h following exposure when 62 schistosomula were found: stratum corneum 18 %, stratum germinativum 72 %, dermis 10 %, and hypodermis 0 % (fig. 5). Sections at day 2 were negative but 13 schistosomula were seen in the stratum corneum of one of 2 ducks at day 4, and 7 in another duck at day 5.

Cercarial bodies were recovered from teased skin of 1 bird each at days 1 and 3, and 1 of 2 birds at day 6 (fig. 4).

Among the birds which were challenged 3 times or more, the skin was found to contain dead schistosomula on days 1, 2, 3, 4, 5 and 7, but was negative thereafter (fig. 6). All of the parasites found in sections of web at day 5 were in the stratum corneum.

After the first challenge exposure, parasites were present in the lung on days 1 - 3, not on day 4, but again on days 5 - 8 and on day 12 (fig. 1). On days 1 and 2, the majority found were located in parabronchi and apparently were adhering to lung tissue (fig. 7). On day 3, none could be seen in the parabronchi but 27 % of those found were in the lumena of secondary bronchi where they were either stuck to the bronchus wall or enveloped in mucus-like material (fig. 8). Significant numbers were found in veins on only one occasion (1 of 2 birds on day 7), and only on days 1 and 8 were worms seen in arteries at all (Table I). After day 1, from 35 - 83 % of all worms in the lung were in lymphoid areas (Table I, see fig. 40).

After second challenge, schistosomula were found from day 1 to 6 and on day 9 (fig. 4) when a single worm was seen. Lung examinations were negative on days 10, 14, 15, and 27 (fig. 4). Histological examination revealed that 88 % of 48 schistosomula in the lung on day 2 occupied parabronchi but 63 % of these were adhering tightly to tissue (fig. 9). Similarly, on day 6, 70 % of the 16 worms in secondary bronchi were adhering to walls of the bronchi and/or were surrounded by mucus-like material (fig. 10). Nineteen percent (day 4)

and 6 % (day 6) were in veins under the epithelium of secondary bronchi, and 8 %, 71 %, and 60 % of the lung parasites on days 2, 4, and 6 were found in lymphoid areas (Table I).

After later challenges schistosomula did not appear in the lung until day 3. They were present on days 4, 5, and 7 but not thereafter (fig. 6). Two of the 4 found in sections of lung on day 7 were stuck to the walls in parabronchi, the other 2 being in lymphoid areas (Table I).

Schistosomula were found in the livers of 16 of 19 birds examined after first challenge (days, 1, 2, 3, 4, 6, 8, and 12) (fig. 1). In fact, most of the worms found on days 2, 4, 5, 8, and 12 were located in the liver where the majority occupied sinosoids and many were surrounded by lymphocytes (Table II, fig. 11). By contrast, only 4 of 15 birds examined after second challenge had parasites in the liver (fig. 4), these being 1 of 1 on day 2, 2 of 3 on day 4, and 1 of 2 on day 6. In each of the cases on days 2 and 4, the liver contained the bulk of the duck's worm burden (Table II) and in all 4 birds, most of the liver parasites were in sinusoids surrounded by lymphocytes. Twenty birds with 2 or more previous exposures were examined but only 3 of these had parasitized livers (fig. 6), 2 being sacrificed 4 days and 1, 7 days after the last challenge. Seventy-nine percent of the 7 day-old worms were in sinusoids and the rest in veins. About 1/3 of those in sinusoids were surrounded by lymphocytes.

The occurrence of worms in the kidney was erratic. After first challenge, for example, less than 5 were found there on days 1 and 8, but 14 of the 86 parasites found on day 2, 50 of 454 on day 3, 100 of 133 on day 4, and 30 of 100 on day 6 were in the kidney. After

second challenge, kidney examinations were negative on days 3, 4, 10, 15, and 27, and after 3 or more challenges there was no renal parasitism in birds killed on days 2, 4, 7, 9, and 23, but worms were found in a second duck sacrificed on day 4. This was the same bird in which worms were found in the liver.

The intestine was examined on days 7, 8, 28, 36, 89, and 99 after first challenge and was negative each time. At day 7 two reactions, typical of those around old eggs, were seen (106 days after initial infection). No worms were found anywhere in a duck's body after day 12 following first challenge. After second challenge, the intestine was negative on days 4, 5, 10 and 15 and, with the exception of a single adult seen at the tip of a villus on day 14, no worms were seen anywhere after day 9 following second challenge. After 3 or more challenges, the intestine was negative at days 7, 8, 9, and 11, and no worms were found anywhere after day 7.

II GROWTH AND MATURATION:

The mean lengths of schistosomula from challenged birds rose from 211 μm on day 1 to a maximum of 610 μm on day 4. The sole specimen yielded by a thorough search of both lungs on day 9 measured 368 μm (Table III, fig. 12). Day 6 and 7 schistosomula are of special interest because, in initial infections, sexual maturity and adult size, averaging about 2500 to 3000 μm, (fig. 13) are reached by this time (Ellis, 1968). The mean lengths of worms recovered from 3 challenged ducks on days 6 and 7 were 286 μm, 318 μm, and 240 μm, respectively (Table III, fig. 14).

The parasite digestive tract appeared to be functional in that 33 - 93 % of worms in the host's lungs and 40 - 100 % of those

in livers, exhibited black cecal contents, presumably containing hematin (Tables I and II). On the other hand, development never proceeded beyond the point where a short posterior extension arose from the cecal junction, and in 2 6-day-old worms, intact, undigested erythrocytes were visible (fig. 15). Further, in 4 other 6-day-old worms, hematin was seen in the parenchyma, implying that the integrity of the gut had been lost.

Among the 19 birds examined after first challenge, only 3 yielded worms which appeared to be maturing sexually. In one of these, the 4-day-old parasites in the liver appeared to be normal but those in the lung were retarded. Ten 6-day-old worms were recovered from a second bird. One of these had a developing gynocophoric canal and 3 showed sperm in the posterior testes. All of the parasites in a third duck appeared to be normal for their age, 8 days. In contrast, almost no sexual development was evident in worms collected from the remaining challenged birds. The tegumental spines of the cercarial stage were still present in virtually all 6 - 7 day-old worms (fig. 16), the genital primordium remained undifferentiated (fig. 17), and in some, even the cercarial head organ (fig. 18) and eye spots were still visible.

III CONDITION:

Reference was made above to the fact that not all worms had hematin-filled caeca, implying that some had not fed recently. The relative numbers of worms with colorless caeca showed no consistent relationship with the site in the host (lung or liver) but for the first 6 or 7 days of infection, they were consistently lower than their counterparts in wholly susceptible birds (Table IV).

Another observation, related to the foregoing only in that it, too, may be an indicator of the state of health of the worms, was that schistosomula seen in sections as well as those recovered whole from teased tissue, were frequently covered by a mucus-like substance with or without host cells attached (fig. 19). The numbers of worms encased in mucoid material and lymphocytes appeared to be greatest during days 1 - 4 of challenge infection and to be higher in the lung than in the liver (Tables I, II). However, the data derived from different birds through time are quite variable and fail to provide convincing patterns of distribution. A comparison of schistosomula from different sites within a single bird, however, (Table V) shows clearly that 3 day-old worms in the skin have grown less than those in the lung or liver, that more worms in the liver have full caeca than those in the lung or the skin, and that almost all of the parasites in the lung were covered by mucoid material and cells while less than half of the liver worms and none of the skin worms were so encumbered.

TABLE I

Distribution of T. ocellata in lungs of challenged birds

DAY	NO. OF CHALLENGES	NO. OF WORMS OBSERVED	% FREE IN ARTERIES	% FREE IN PARA-BRONCHI	% STUCK TO TISSUE IN PARABRONCHI	% FREE IN 2nd BRONCHI	% STUCK TO 2nd BRONCHIAL WALLS OR IN MUCUS	% IN VEINS UNDER 2nd BRONCHI	% IN LYMPHOID AREAS	% FEEDING
1	1	13	8	0	77	0	0	0	15	--
2	1	26	0	35	30	0	0	0	35	66
2	2	48	0	33	55	4	0	0	8	79
3	1	45	0	2	0	0	27	4	67	33
4	2	21	0	5	0	0	5	19	71	88
5	2	1	0	0	0	0	100	0	0	--
6	2	50	2	0	0	10	22	6	60	50
7	3	4	0	0	0	0	50	0	50	50
7	1	41	0	0	2	0	0	29	69	82
8	1	66	6	11	0	0	0	0	83	93

TABLE II

Distribution of T. ocellata in liver of ducks

after 1st, 2nd and 3rd challenges

DAY	CHALLENGE NUMBER	DAYS SINCE PREVIOUS EXPOSURE	NUMBER OF WORMS OBSERVED	% FREE IN SINUSOIDS	% IN SINUSOIDS SURROUNDED BY LYMPHOID CELLS	% IN VEINS	% FEEDING
1	1	35	0	--	--	--	--
2	1	420	182	23	75	2	78
2	2	--	161	22	73	5	38
3	1	86	77	3	97	0	62
4	2	--	87	10	83	7	67
6	2	46	84	36	26	38	40
7	3	46	52	50	29	21	60
7	1	106	25	60	4	36	100
8	1	850	117	21	5	74	100

TABLE III

Mean lengths of T. ocellata schistosomula recovered from lungs or livers of ducklings after 1 - 9 days in initial and challenge infections

DAYS SINCE LAST EXPOSURE TO CERCARIAE	INITIAL INFECTIONS		CHALLENGE INFECTIONS		P*
	N	MEAN LENGTH (μm)	N	MEAN LENGTH (μm)	
1	44	237 ± 5**	16	211 ± 7	<0.01
2	16	300 ± 11	14	260 ± 8	<0.01
3	53	572 ± 11	24	295 ± 18	<0.001
4	24	1420 ± 34	9	427 ± 34	<0.001
4			45	325 ± 7	<0.001
4			13	610 ± 22	<0.001
6	5	1484 ± 22	29	286 ± 11	<0.001
6			21	318 ± 19	<0.001
7	8	2795 ± 222	8	240 ± 14	<0.001
9	80	2720 ± 50	1	386	

* Probability derived from Student's "t" test

** Standard error

TABLE IV

Occurrence of T. ocellata with hematin-filled caeca in lungs and livers of ducklings with primary and challenge infections

DAYS SINCE LAST EXPOSURE TO CERCARIAE	INITIAL INFECTION				CHALLENGE INFECTION			
		LUNG		LIVER		LUNG		LIVER
	N	% FEEDING	N	% FEEDING	N	% FEEDING	N	% FEEDING
1	60	75%	11	9%				
2	25	100%	10	90%	26(1)*	66%	182	78%
2					48(2)	79%	161	38%
3					45(1)	33%	77	62%
4	5	100%	59	97%	21(2)	88%	87	67%
5	7	100%	26	100%				
6	22	100%	36	97%	50(2)	50%	84	40%
7	12	100%	26	96%	4(3)	50%	52	60%
7					41(1)	82%	25	100%
8					66(1)	93%	117	100%
9	60	100%	16	100%				

* Number of previous exposures in brackets

TABLE V

Distribution, size, and condition of schistosomula in a duck 3 days after first challenge

LOCATION	NO. WORMS RECOVERED	MEAN LENGTH (μm)	% WITH GUT CONTENTS	% WITH MUCOID AND CELLULAR COVERING
Liver	24	0.295	50	41
Lung	18	0.263	20	94
Skin	7	0.165*	0	0

* $P < 0.01$

IV ULTRASTRUCTURE OF THE TEGUMENT AND GUT OF T. OCELLATA:

A decision was made to find out if physical lesions were evident in the bodies of T. ocellata from challenge infections. Prior to doing this, however, it was necessary to determine the normal ultrastructure for the species, this being entirely unknown.

It was found that, like other trematodes, T. ocellata is ensheathed in a continuous, living layer of cytoplasm which has been termed a "tegument" (Threadgold, 1963).

The tegument was bounded distally by a typical trilaminate plasma membrane approximately 10 nm in diameter although there were infrequent indications of a more complicated, multilaminate structure (fig. 20). Invaginations in this membrane produced many tubular and vacuolar profiles which increased in number and complexity as the worm matured (see fig. 32). Proximally, the tegument was bounded by a basement membrane which was continuous with cytoplasmic processes connecting the tegument to nucleated regions within the parenchyma (fig. 21). These cytoplasmic processes were supported internally by microtubules (fig. 21). Each "cyton" contained a single nucleus usually with one nucleolus and prominent chromatin granules (fig. 21 and 22). The cytoplasm contained mitochondria, rough endoplasmic reticulum and Golgi apparatus (fig. 22). Intercellular spaces or cavernous cisternae (Bruce et al., 1970) contained large lipid-like granules (370-820 nm) (fig. 21) which were often accompanied by mitochondria. Besides spines (fig. 22 and 23) which, when sectioned longitudinally, revealed alternating election-opaque and election-lucent bands with an interband period of about 12 nm (fig. 24), rod-like inclusions 130-160 nm x 20-40 nm, and mitochondria, the

tegument contained two types of vesicular profiles:

(i) Dense bodies 130-300 nm which originated in the cytoplasm of tegumental cytons (fig. 22) and were seen in cytoplasmic (internuncial) processes as well as the tegument proper (figs. 22 and 23).

(ii) Laminated vesicles 170-350 nm in diameter and were often seen in association with invaginations of the superficial plasma membrane and were seen within internuncial processes. Contents included amorphous, fibrillar and granular material (fig. 25).

Separating the syncytial tegument from the nucleated region below were, first, a fibrous layer (fig. 23) and then two muscle layers, an outer circular one and an inner longitudinal one (figs. 22 and 23).

The external tegument was continuous with the lining of the oesophagus which in turn was surrounded by two muscle layers, a closely apposed circular layer, and an outer, longitudinal layer (fig. 26). Nucleated regions lay beyond the muscle layers and were joined to the "tegumental" lining by cytoplasmic processes similar to those joining the external tegument. The oesophageal tegument included mitochondria and vesicular profiles similar to those described above in the tegument proper.

Posterior to the caecal bifurcation the gut was lined by a nucleate, syncytial epithelium, the cytoplasm of which contained numerous mitochondria and rough endoplasmic reticulum (fig. 27). Frequent sheet-like lamellae extended from the surface and projected a short distance into the lumen (fig. 28) some apparently "engulfing" lumenal contents.

The basal plasma membrane was separated from a fibrous layer

below by a very thin basal lamina (fig. 27). Outside the fibrous layer were circular and longitudinal small muscle bundles. The basal plasma membrane was thrown into long invaginations which penetrated much of the depth of the epithelium (fig. 27).

Although incompletely digested white cells were seen in the lumen of the intestine (fig. 27), red cells were never identifiable, indicating immediate lysis upon entry into the oesophagus. Numerous lipid-like droplets could be seen in the lumen as well as within the epithelium itself. Some of these droplets had a dense ring confined to the periphery, while this densification included more of some and less of other droplets (fig. 27). There were also profiles of uniformly more election dense droplets, most being in contact with the epithelium (fig. 27). The most common profile within the lumen was that of concentric rings of what are probably cell membranes from lysed cells (fig. 27).

Worms recovered from challenged ducks showed surprisingly little difference over those from normal ones at days 3, 5, 6, and 9. There was a noticeable increase in the density of the tegument in some areas especially the superficial plasma membrane (figs. 29 and 30). Figure 30 was of special interest since it represented a worm after 9 days in an immune environment. The concentration of dense bodies in the tegument and the presence of spines and rod-like inclusions seen here were typical of day 2 or 3 initial infection worms (spines and rod-like inclusions were not seen in adults). The tegument did appear to be losing its integrity in places (fig. 31).

V PEROXIDASE TECHNIQUE - LOCALIZATION OF ANTIBODIES:

An effort was made to determine, at the ultrastructural

level, if and where humoral antibody in immune ducks becomes bound to invading parasites.

Only 2 worms were recovered from the hyperimmune bird used in this experiment. Both of these, however, when treated with peroxidase-labelled rabbit anti-duck gamma globulin showed accummulations of electron-dense particles on the superficial plasma membrane of the tegument (fig. 33).

Three worms from an initial infection treated similarly and a further control not reacted with rabbit anti-duck serum but treated for the presence of peroxidase, were all negative (fig. 34).

Cristae and outer membranes of mitochondria in worms from all birds were selectively electron-dense.

VI INTEGRITY OF PLASMA MEMBRANES - BENZPYRENE TECHNIQUE:

If the normal structure of the plasma membrane (phospholipid layer sandwiched between two protein layers) is sufficiently deformed by the binding of antibody, the phospholipids become unmasked and the damaged membrane will bind lipid dyes or fluorochromes (bitensky, 1963). Thus, the relative "fatiness" of membranes becomes a measure of the damage done to them and can be assessed by noting the amount and distribution of fluorescence seen in ultraviolet light.

Worms were chosen 3 - 5 days after exposure for this procedure because it was known that "challenge" worms were already affected in terms of growth by this time, and that they and initial infection worms were reasonably accessible in lung or liver.

The gut and/or tegument and parenchyma of 58 - 100% of worms retrieved from challenged birds fluoresced, indicating damage to cell membranes involved (Table VI and fig. 35).

There was no discernible fluorescence in worms from 4 of 5 primary-infection ducks. Worms from the fifth bird showed some fluorescence in the gut only (fig. 36 and Table VI).

In an attempt to assess the possible damage done to worms by cells from an immune duck, 240 x 10^6 and 339 x 10^6 viable spleen cells from a hyperimmune duck were injected into two normal ducklings before they were exposed to cercariae. As a control, 432 x 10^6 normal spleen cells were injected (i.p.), into a third duckling.

None of the schistosomula retrieved from these birds 3 days later revealed any fluorescence when treated with benzpyrene.

TABLE VI

Condition of plasma membranes of T. ocellata from primary and challenge infections as revealed by the benzpyrene technique

DUCK	DAYS SINCE LAST EXPOSURE	NO. WORMS RECOVERED	RESULTS
B-7764	3 (P)*	13	4/13 - gut fluoresced.
W-364	3 (C)*	21	17/21 - gut fluoresced - some diffuse fluorescence in parenchyma and teg.
B-19517	4 (P)	10	No fluorescence
B-100	4 (C)	12	7/12 gut fluoresced, some diffuse fluorescence in teg.
B-612	4 (P)	35	No fluorescence
B-2781	4 (P)	4	No fluorescence
B-69	4 (C)	5	Gut and anterior and posterior ends fluorescent
B-7793	5 (P)	22	No fluorescence
B-61	5 (C)	1	Lining of gut fluoresced.

*P = Primary Infection
*C = Challenge Infection
teg = Tegument

VII IMMUNOSUPPRESSION:

The role of the immune response in limiting primary infections or in resisting challenge infections was investigated by attempting to suppress the immune response by subjecting ducks to varying doses of irradiation.

Four ducks exposed to more than 800 rad died within 12 h. Among those that survived, there was a rough correlation between the level of irradiation and the reduction in circulating leucocyte levels (Table VII). Nine to 18 days after irradiation the ducks were exposed to cercariae and the numbers of parasite eggs, represented by hatched miracidia were determined over 24 h. periods (fig. 37). In each case a peak was established by day 18 - 20 and by day 40 egg laying was reduced to insignificant levels (less than 5 miracidia/day).

After re-exposure to cercariae, 8 of 9 birds showed no evidence of a second infection, the sporadic passage of very small numbers of eggs (Table VIII) being attributed to the primary infection (Rau, 1969). One bird (# 344), however, passed significant though low, numbers (peak 68 on day 20, Table VIII) suggesting that a second infection had become established.

TABLE VII

Effects of irradiation and subsequent infection by

T. ocellata on circulating leucocyte levels

in White Pekin ducklings

DUCK	RADIATION (RADS)	LEUCOCYTES PER CU.M.M. I	AGE (DAYS) WHEN EXPOSED TO CERCARIAE	LEUCOCYTES PER CU.M.M. II	INCREASE IN LEUCOCYTES/ DAYS SINCE LAST DETERMINED
212	nil	48750(9)*	n.e.	47750(13)*	--
213	nil	39250(9)	n.e.	34500(13)	--
349	nil	20500(8)	n.e.	20000(24)	--
348	nil	38000(8)	6	56750(24)	18750/16
206	400(3)*	8750(9)	13	10750(13)	2000/4
208	400(3)	8750(9)	n.e.	9750(13)	1000/4
338	500(2)	7750(9)	20	25000(20)	17250/11
339	500(2)	--	20	17500(20)	--
340	600(2)	6750(9)	11	41000(24)	34250/15
372	600(2)	--	14	24000(24)	--
342	700(2)	3000(9)	11	31500(24)	28500/15
343	700(2)	--	14	58000(24)	--
344	800(2)	4500(8)	11	22000(24)	17500/16
345	800(2)	7500(8)	14	32000(24)	24500/16
202	900(3)	died(3)	--	--	--
210	900(3)	died(3)	--	--	--

* - Age, in days, of duck

n.e. - not exposed

TABLE VIII

Numbers of miracidia collected on the days indicated after second exposure to cercariae of *T. ocellata*

DAYS SINCE RE-EXPOSURE	DUCK NO.								
	338	339	340	372	342	343	344	345	206
13	0	2	9	3	2	0	22	0	11
14	0	-	1	-	0	-	8	-	-
15	3	-	0	-	1	-	27	-	-
16	-	0	-	0	-	6	-	0	1
17	-	0	-	0	-	-	-	0	1
18	3	0	-	0	-	2	10	0	5
19	0	2	6	0	8	1	65	0	5
20	0	-	3	-	3	-	68	-	-
21	0	0	0	0	0	0	28	0	0
22	-	0	0	0	-	0	-	0	0
23	-	-	-	-	-	-	-	-	0
25	1	-	0	-	0	-	0	-	-
26	0	-	0	-	0	-	0	-	-
27	1	-	-	-	0	-	0	-	-
28	0	-	0	-	1	-	1	-	-

VIII ADOPTIVE AND PASSIVE TRANSFER:

White pekin ducklings were injected with spleen cells, Meckel's diverticulum cells, serum, or cells plus serum, derived from donor birds which were immune to reinfection by T. ocellata. Ducklings receiving cells and/or serum from susceptible birds or saline and untreated ducklings, served as controls. The birds were then exposed to cercariae and the success or failure of the parasite challenge (i.e. the failure or success of the transfer of immunity) was judged by either assessing growth and development of schistosomula recovered from birds killed after 3 - 5 days, or by counting the numbers of viable eggs passed with the feces of birds which were kept alive. Among the collections of schistosomula, one case produced only worm fragments, in the others, the numbers of measureable worms varied from 2 - 70.

1. SPLEEN CELLS: The worms recovered from the experimental birds 3 - 5 days after exposure were not significantly different from those found in the control ducks (Table IX). In one case, the untreated control produced worms which were larger than those from the experimental or the normal-treated control birds, in another, the schistosomula from the normal-treated were longer than those from the hyperimmune-treated, but this particular pair had no untreated control partner.

2. MECKEL'S DIVERTICULUM CELLS: Similarly, worms from recipients of Meckel's diverticulum cells taken from hyperimmune donors were not significantly different from those found in control birds, the only exception being one case in which the experimental bird produced worms which were actually longer than those from the untreated control (Table X).

3. SPLEEN CELLS PLUS SERUM: No significant differences were seen between the lengths of worms from the experimental birds and those from the dual controls except in one anomalous case when the experimental actually produced longer worms than did either of the controls (Table XI). It was noted that in 3 experiments, the experimental's worms were smaller than the normal-treated control's were, but not significantly different from the untreated control's parasites. This might be rephrased to state that in 3 cases the normal-treated control's parasites were significantly larger than those from either the experimental or the untreated control birds.

4. SERUM: Again, parasites were recovered from the lungs of all experimental and control birds. When passive transfer was attempted by means of injecting serum alone no differences were found between experimental and control groups when only 5.5 ml of serum was injected, but among the birds given 6.0 and 8.0 ml of serum, schistosomula from the hyperimmune-treated ducklings were significantly shorter than those from the normal-treated birds, and shorter, though not significantly so in both cases, than those from the untreated birds (Table XII). The three birds which were kept alive passed hundreds of viable parasite eggs on day 20 (Table XII). When massive amounts of serum were employed in transfer experiments, schistosomula from hyperimmune serum recipients were consistently shorter than those from birds which received either normal serum or sterile saline, although small sample size denied statistical significance in two cases (Table XIII). No significant differences were detected between the two controls in any of the experiments. A second massive volume experiment was performed in

which 78 ml of hyperimmune serum, normal serum or saline were injected into ducklings over a 12 day period. The birds were exposed to cercariae when one day old and feces were examined for parasite eggs by the miracidial hatch method beginning on day 8, continuing until day 25, and once more on day 30, except for the fact that feces of 8 of the 9 control birds were not examined for 4 or 5 days between days 19 and 23. All control birds developed patent infections, whereas one of the hyperimmune serum-treated ducklings failed to pass a single parasite egg and a second passed one lone egg on day 25 (Table XIV, Fig. 38). Also, whereas birds among the control groups began to pass viable eggs as early as day 11, patency in the experimental group was first seen on day 16. It is unfortunate that data are incomplete for the control groups (fig. 38) because complete data would render more obvious the difference between controls and hyperimmune serum-treated birds. The total number of eggs passed by the 5 hyperimmune serum-treated birds whose feces were examined on each of the 19 test days, was 47. By contrast, the 4 saline-treated birds together produced 410 eggs, with 5 days' data missing for each duck, and the 5 normal serum-treated birds produced 194 eggs, with 4 days' data missing for 2 birds and 5 days' data missing for another.

TABLE IX

Mean lengths of 3–5 day old *T. ocellata* from untreated ducklings and from birds previously injected with spleen cells from immune and susceptible donors

DONOR DUCK NO.	DAYS SINCE DONOR EXP. TO CERCARIAE	RECIPIENT DUCKLING NO.	NO. VIABLE CELLS INJECTED	NO. WORMS RECOVERED	AGE OF WORMS (DAYS)	MEAN LENGTHS OF WORMS (um)		
WP52	8	443	487×10^6	23	4	472		
WP412	-	446	312×10^6	18	4	585	$p>0.05$	$p>0.2$
None	-	476	-	8	4	527	$p>0.5$	
WP52	8	444	597×10^6	7	5	408		
WP412	-	447	156×10^6	21	5	505	$p>0.2$	$p>0.05$
None	-	482	-	8	5	569	$p>0.4$	
WP15	10	112	66×10^6	26	4	154*		
WP407	-	111	26×10^6	21	4	152*	$p>0.5$	$p<0.005$
None	-	117	-	18	4	202*	$p<0.01$	

TABLE IX (Cont'd)

WP15	10	113	60 X 10^6	11	5	219‡		
WP407	-	110	42 X 10^6	0*	5	-	-	$p > 0.5$
None	-	118	-	4	5	214‡	-	
WP12	17	1050	564 X 10^6	35	3	243		
WP421	-	1046	120 X 10^6	56	3	319	$p < 0.001$	
WP38	17	1044	687 X 10^6	2*	5	911		
WP427	-	1040	729 X 10^6	2*	5	1055	$p > 0.5$	
WP53	30	186	339 X 10^6	12	4	149‡		
WP408	-	109	13 X 10^6	11	4	154‡	$p > 0.5$	$p > 0.2$
None	-	71	-	18	4	160‡	$p > 0.5$	

* Maturing fragments found. Qualitative indication of normal growth.

‡ Anterior end to ventral sucker measurement.

TABLE X

Mean lengths of 4-5 day old *T. ocellata* from untreated ducklings and from birds previously injected with Meckel's Diverticulum cells from immune and susceptible donors

DONOR DUCK NO.	DAYS SINCE DONOR EXP. TO CERCARIAL	RECIPIENT DUCKLING NO.	NO. VIABLE CELLS INJECTED	NO. WORMS RECOVERED	AGE OF WORMS (DAYS)	MEAN LENGTHS OF WORMS (um)		
WP52	8	445	30×10^6	20	4	613		
WP412	-	448	10×10^6	17	4	551	$p>0.2$	$p<0.001$
None	-	477	-	34	4	458	$p<0.005$	
WP433	8	490	6.3×10^6	10	5	872		
WP405	-	486	42×10^6	1*	5	1119	-	$p>0.05$
None	-	492	-	11	5	831	-	
WP12	17	1049	?	14	4	540		
WP421	-	1045	?	33	4	474	$p>0.05$	$p>0.5$
None	-	99	-	10	4	516	$p>0.03$	

TABLE X (Cont'd)

WP38	17	1043	20×10^6	3	5	597
WP427	-	1042	20×10^6	1	5	767

* Maturing fragments found. Qualitative indication of normal growth.

TABLE XI

Mean lengths of 4-5 day old T. ocellata from untreated ducklings and from birds previously injected with spleen cells and serum from immune and susceptible donors

RECIP. NO.	CELL DONOR NO.	DAYS SINCE EXP.	NO. OF VIABLE CELLS	ML SERUM INJ. EA. OF DAYS (AGE OF BIRD)	RECIP. EXP. @ AGE**	NO. & (AGE)** OF WORMS RECOVERED	MEAN LENGTHS OF WORMS (um)		
487	422	8	414 X 10^6	2.0 (3&8)	7	51 (4)	343		
483	405	-	972 X 10^6	2.0 (3&8)	7	38 (4)	434	p<0.001	p>0.5
491	None	-	-	-	7	51 (4)	347	p<0.001	
488	422	8	348 X 10^6	2.0 (3&8)	7	6 (5)	666		
484	405	-	678 X 10^6	2.0 (3&8)	7	6 (5)	468	p<0.025	p>0.2
492	None	-	-	-	7	11 (5)	831	p<0.025	
496	119	7	?	2.0 (6&7&8&10)	8	70 (4)	283		
498	426	-	393 X 10^6	2.0 (6&7&8&10)	8	29 (4)	420	p<0.001	p>0.1
474	None	-	-	-	8	9 (4)	310	p<0.005	

TABLE XI (Cont'd)

497	119	7	?	2.0 (6&7&8&10)	8	24 (5)	394		
499	426	-	420 X 10^6	2.0 (6&7&8&10)	8	8 (5)	696	$p < 0.001$	$p > 0.4$
475	None	-	-	-	8	5 (5)	448	$p < 0.025$	
459	410	9	119 X 10^6	2.0 (2&3&5)	8	15 (4)	412		
457	406	-	336 X 10^6	2.0 (2&3&5)	8	11 (4)	389	$p > 0.5$	$p > 0.2$
463	None	-	-	-	8	4 (4)	519	$p > 0.2$	
460	410	9	162 X 10^6	2.0 (2&3&5)	10	0 (5)	*		
458	406	-	528 X 10^6	2.0 (2&3&5)	10	0 (5)	*	-	-
464	None	-	-	-	10	0 (5)	*	-	

* Maturing fragments found. Qualitative indication of normal growth.

** Age in days

TABLE XII

Effects of serum from normal* and hyperimmune** donors on the growth and reproduction of T. ocellata in ducklings

"A" GROWTH

RECIP. NO.	SERUM	MLS. INJ. ON DAY (AGE) OF BIRD	RECIP. EXP. @ AGE (DAYS)	NO. & AGE (Days) OF WORMS	MEAN LENGTHS (μm) OF WORMS	
435	**H	1.5(2), 2(6), 2(14)	12	7(5)	594	
						p>0.5
436	*N	1.5(2), 2(6), 2(14)	12	9(5)	561	
440	H	1.5(2), 2(6), 2(14)	12	24(4)	379	
439	N	1.5(2), 2(6), 2(14)	12	28(4)	358	p>0.5
187	H	2(8), 2(10), 2(13)	11	7(5)	521	
						p<0.025
190	N	2(8), 2(10), 2(13)	11	11(5)	630	p>0.2
						p>0.5
193	None	-	11	14(5)	610	
188	H	2(8), 2(10), 2(13), 2(18)	17	40(4)	323	
						p<0.001
191	N	2(8), 2(10), 2(13), 2(18)	17	40(4)	392	p=0.09
						p<0.01
194	None	-	17	40(4)	348	

TABLE XII (Cont'd)

		"B" REPRODUCTION		NO. MIRACIDIA ON DAY 20
432	H	1.5(2), 1(6), 2(14)	12	5000
434	H	2(2), 2(6), 2(14)	12	12000
442	H	2(2), 2(6), 2(14)	12	2000

* Normal - Adult White Pekin with no exposures to T. ocellata

** Hyperimmune - Adult White Pekin with 3 or more exposures to T. ocellata

TABLE XIII

Body lengths of *T. ocellata* schistosomula after 4 days in ducklings injected at 12 hour intervals with hyperimmune or normal serum or saline beginning when birds 1 day old

BIRD NO.	RECEIVED	TOTAL VOL.	BIRD'S AGE WHEN EXP. TO CERCARIAE	NO. WORMS RECOVERED	MEAN LENGTH (um)		
239	HI serum	28.5 ml	2 days	22	374		
						< 0.001	
243	N serum	"	"	6	624		< 0.001
						NS	
247	saline	"	"	10	617		
240	HI serum	37.8 ml	3 days	8	457		
						NS	
244	N serum	"	"	2	557		< 0.05
						NS	
248	saline	"	"	5	668		
241	HI serum	44.8 ml	4 days	3	431		
						< 0.005	
245	N serum	"	"	12	685		NS
						NS	
249	saline	"	"	3	675		

TABLE XIII (Cont'd)

242	HI serum	60.0 ml	5 days	32	428		
246	N serum	"	"	10	627	<0.01	<0.05
250	saline	"	"	12	556	NS	
Total	HI serum	----------		65	413		
"	N serum	----------		30	645	<0.001	<0.001
"	saline	----------		30	610	NS	

eventually involving polymorphs, eosinophils, lymphoid cells, and large mononuclear cells. Dead and dying worms were seen in these foci. Although there was an increased cellular response in lungs of resistant ducks to T. ocellata, it appeared to be more diffuse and more predominantly lymphoid than that described by Lichtenberg and Ritchie (1961). Only after worms became moribund did it involve large numbers of heterophils and other granular leucocytes.

Similar retention of schistosomula in lungs of experimental hosts has been reported by Vogel and Minning (1953) and Davis et al. (1963) (S. japonicum in Macaca mulatta) by Lin and Sadun (1959) (S. japonicum in mice, rabbits, and monkeys) and by Olivier and Schneidermann (1953) and Magalhaes (1959) (S. mansoni in mice) among others. The latter study showed that in initial infections worms could not be found in the lung after day 8 whereas in challenge infections schistosomula were recovered from the lungs as late as day 40, most of them dead or apparently dying. Similarly, using isotopically labelled cercariae, Lewert and Para (1969) showed that rates of migration and passage through lung were markedly slower.

Although Lichtenberg and Ritchie (1961) reported an albuminoid material being exuded into the alveoli of challenged mice, the relationship between that and the observed "mucoid secretions" in bronchial branches in the present work is hard to assess. The significance of its presence and its effects on the migrating worms may relate to physically slowing down the migratory process and allowing sensitized cells to "home in on their target" (Lichtenberg, 1967). Newsome (1962) in an in vitro study, showed that opsonizing factors may

DISCUSSION

Stirewalt (1963) and Smithers and Terry (1969a) suggested that it would be useful to augment the classical list of criteria used to identify and evaluate cases of acquired resistance to infection. Accordingly, the present study incorporates both commonly used criteria e.g. duration of infection and size and maturity of worms, as well as some of the less widely used ones such as distribution of worms in host, length of prepatent period, and effectiveness of the skin barrier. Since there was almost no pathology or mortality attributable to initial infections in ducks, criteria based on these parameters were inapplicable.

1 MIGRATION:

Penetration by cercariae of bird schistosomes into mammalian skin has been studied extensively because of the disease-producing results known as "swimmer's itch". Olivier (1949) first established the immunological basis for this syndrome and Stirewalt (1966) noted similarities between the cellular reactions in abnormal host skin and those of normal but resistant hosts upon challenge.

Web skin presented virtually no barrier to schistosomula of T. ocellata in initial infections, none being found in that site after 24 h (Ellis, 1968; Bourns et al., 1973). That this site represented a more significant barrier to parasites of subsequent

exposures is indicated by their being found there in the present work for as long as 3 - 7 days (Figs. 1, 4, and 6). Another indication of impeded migration was the distribution of schistosomula within the skin. In initial infections, only 5 % of the schistosomula remained in the stratum corneum after 8 h and by day 1 all had penetrated to deeper layers (Ellis, 1968). In contrast, the stratum corneum continued to contain the majority of worms in the skin on days 1 through 5 in challenged birds. As early as 10 h the discrepancy between initial and challenge infections was apparent. Only 2 % remained in the stratum corneum after 10 h in initial infections and 76 % had moved into or below the dermis (Bourns et al., 1973). In contrast, after 10 h of a second challenge 18 % remained in the stratum corneum and only 10 % had penetrated into the dermis.

Since all schistosomula revealed in sections of skin after day 2 were in the stratum corneum and since worms did arrive in the lungs, it seems that only those which were unable to breach the cornified layers were permanently trapped in the skin. However, the integrity of those trapped in lower layers of the skin might have been lost quickly due to cellular infiltration and phagocytosis, making them difficult to recognize.

An early, heightened cellular response to cercarial challenge in mice previously exposed to Schistosomatium douthitti was reported by Kagan and Meranze (1955) who also noted that significant numbers of worms reached the lung despite an increased inflammatory response in the skin. Lichtenberg and Ritchie (1961) reported marked differences between the duration and severity of cercarial dermatitis in immune rhesus monkeys and in normal controls

when exposed to cercariae of S. mansoni. At the peak of this reaction (24 - 48 h) they observed erosion and massing of polymorphonuclear leucocytes in the epidermis, followed later by hyperkeratosis, crust formation, and scaling. The latter two symptoms were also seen in the present work when the exposed foot was crusty and scaling 2 - 3 days after exposure while the unexposed one retained its normal, soft texture. Despite the cellular response, Lichtenberg and Ritchie (1961) reported no differences in the speed of cercarial progression from epidermis to dermis, nor in the number of cercariae persisting in the skin after 5 days. These authors reported that most larvae which were permanently trapped in the skin remained in the dermis. Clegg and Smithers (1968), on the other hand, working with S. mansoni in rats, reported that most deaths occurred in the first 10 min of penetration, before the schistosomula reached the dermis. Up to 50 % of cercariae died during penetration of normal rat skin and there was no significant increase in mortality in immune rats.

In an experiment with S. mansoni in 4 rhesus monkeys, however, Clegg and Smithers (1968) noted that cercarial mortality rates in 2 hyperimmune monkeys were higher than in 2 controls 15 min and 24 h after exposure. In one of the hyperimmune animals the increase in per cent death between 15 min and 24 h was much greater than in either control (from approximately 54 - 85 % vs. from approximately 31 - 46 %, respectively). This result, though limited, is interesting in relation to the present work since the rhesus - S. mansoni system and the duck - T. ocellata system are the only 2 known to develop complete resistance to challenge after allowing a normal initial infection to develop.

Lin and Sadun (1959) presented evidence from rabbits, mice, and monkeys infected with S. japonicum, that skin represented a significant barrier to penetrating cercariae of this species in that their migration was slowed. For example, arrival in the lung was delayed from 16 h in initial infections to 2 days in mice challenged more than 2 months after the sensitizing exposures. The intervals between initial and challenge exposures were long enough to eliminate hyperkeratotic skin reactions (Stirewalt, 1953, 1958) as explanations for the delay.

Although it is tempting to attribute the increased trapping and slowed migration to an acquired immune response, unequivocal evidence in support of that contention is difficult to secure. It is known that resistance to penetration increases with age (Lewert and Lee, 1954; Lewert and Mandlowitz, 1963) and although injecting immune serum directly into the path (skin) of penetrating cercariae reduced worm burdens in rats (Ogilvie, 1964), this technique was not effective in rhesus monkeys, more normal hosts (Ogilvie et al. 1966). Ogilvie and Jones (1969) later considered that this anaphylactic-type antibody did not contribute to protective immunity in the rat. Therefore, the present results must be interpreted cautiously in terms of the immune response.

In initial infections, there were two periods when the lungs contained most of the worms: days 1 - 3, after arrival from the skin, when 60 - 95 % were found in air spaces and secondary bronchi (Ellis, 1968) and days 8 - 9 when, as adults, they returned from the liver and 75 - 95 % were located in arteries (see Fig. 39). No such consistent pattern emerged from the present study of challenge infections (Figs. 1,

4, 6). Schistosomula were later arriving in the lung, they were retained there longer, and having left, apparently never returned (arteries never contained large numbers).

Histology of the lung revealed marked differences also. On days 1 - 3, although most worms from either initial or challenge infections occupied air spaces, a significant number of those in challenge infections were adhering to lung tissue (Table I, Fig. 7). This phenomenon was not observed in initial infections, there often being a clear area, probably a fixation artifact, around individual schistosomula in tissue sites (Ellis, 1968). Secondly, with the exception of one bird in initial infections, worms were virtually absent from the lumina of bronchi after day 4. In challenged birds, on the other hand, up to 50 % of the schistosomula were found in this site as late as day 7 (Table I). Thirdly, in all birds on days 5, 6 and 7 of initial exposures, most worms were in veins, indicating that they were leaving the lung. In only 2 of the challenged birds (days 4 and 7, Table I) were there any indications of worms leaving the lungs. Finally, almost no schistosomula were seen in lymphoid areas until day 6 in initial infections whereas there was an immediate and sustained lymphoid response to worms in the lungs of challenged birds (Table I, Fig. 40).

Lichtenberg and Ritchie (1961), in a study of S. mansoni in rhesus monkeys, showed that trapping of migrating schistosomula occurred mainly in the lungs. There was little or no cellular reaction in the lungs of initially-exposed monkeys, but in resistant monkeys, every schistosomulum became the centre of an inflammatory response,

eventually involving polymorphs, eosinophils, lymphoid cells, and large mononuclear cells. Dead and dying worms were seen in these foci. Although there was an increased cellular response in lungs of resistant ducks to T. ocellata, it appeared to be more diffuse and more predominantly lymphoid than that described by Lichtenberg and Ritchie (1961). Only after worms became moribund did it involve large numbers of heterophils and other granular leucocytes.

Similar retention of schistosomula in lungs of experimental hosts has been reported by Vogel and Minning (1953) and Davis et al. (1963) (S. japonicum in Macaca mulatta) by Lin and Sadun (1959) (S. japonicum in mice, rabbits, and monkeys) and by Olivier and Schneidermann (1953) and Magalhaes (1959) (S. mansoni in mice) among others. The latter study showed that in initial infections worms could not be found in the lung after day 8 whereas in challenge infections schistosomula were recovered from the lungs as late as day 40, most of them dead or apparently dying. Similarly, using isotopically labelled cercariae, Lewert and Para (1969) showed that rates of migration and passage through lung were markedly slower.

Although Lichtenberg and Ritchie (1961) reported an albuminoid material being exuded into the alveoli of challenged mice, the relationship between that and the observed "mucoid secretions" in bronchial branches in the present work is hard to assess. The significance of its presence and its effects on the migrating worms may relate to physically slowing down the migratory process and allowing sensitized cells to "home in on their target" (Lichtenberg, 1967). Newsome (1962) in an in vitro study, showed that opsonizing factors may

be involved in this process.

The fact that this mucoid material was similar in appearance to the contents of the goblet cells indicates a possible role of secretory IgA in the immune response. As early as 1939 Ackert attributed resistance of birds against Ascaridia to the presence of thermostable growth-inhibiting substances in the mucosecretion of goblet cells of the intestinal tract, and Mulligan (1968) pointed out that workers were becoming more interested in the possible role of IgA in immunity of rats to Nippostrongylus brasiliensis. Moreover IgA is known to be the predominant type of immunoglobulin in human bronchial fluids (Tomasi et al., 1963). In this connection, the use of cytochemical techniques to elucidate the distribution of IgA among duck tissues might be very profitable.

The number of ducks in which the liver became parasitized is also an indication of the efficacy of the lung barrier in resistant birds. In initial infections 37/40 (93%) of the birds examined after day 1 had schistosomula or adults in the liver (Fig. 39). This percentage fell to 84 % in first challenges, 29 % in second challenges and 15 % after 3 or more challenges (Figs. 1, 4, and 6). Histology of the liver revealed heightened cellular responses in that organ as well. In contrast to initial infections where there was almost no reaction in the liver until day 6 or later (Ellis, 1968) (See Fig. 41), 73 - 97 % of the parasites seen in the liver on days 1 - 4 of challenge infections were surrounded by lymphocytes and large mononuclear cells (Table II), and fewer had hematin in their ceca (Table IV).

Days 7 and 8 (Table II) were anomalous in that very few of the worms were objects of a cellular response and all of them had hematin-

filled ceca - typical of first infections. Since in most resistant birds it is impossible to find any worms at all by the end of the first week, when worms are found, the immunological competence of the host becomes a matter of question. It is known in the case of the day 8 bird (Table II) that the initial infection involved very small numbers of cercariae (from only three snails) and that few eggs were passed during the patent period. It is probable, then, that the initial infection provided too little antigenic information to provoke an effective immune response (Lineen, 1963). The worms observed evoking little cellular response at day 7 (Table II) probably represent worms from the initial infection (106 days previously) since these were normal adults and severely stunted schistosomula were retrieved from the lungs of the same bird.

The histopathology of the liver in mammalian infections has been studied by several groups of workers (e.g. Lin and Sadun, 1959, Schistosoma japonicum in mice, rabbits and monkeys; Kagan and Meranze, 1957, Schistosomatium douthitti in white mice; Lichtenberg and Ritchie, 1961, Schistosoma mansoni in rhesus monkeys). Generally it has been found that there is little or no cellular reaction early in the initial infection but that after several weeks a periportal accumulation of leucocytes (mononuclears, predominantly) begins - presumably in response to metabolic products (Winslow, 1967).

The reaction of mammals to worms of the challenge infection is usually obscured by the pervasive reaction against eggs of the initial infection in liver parenchyma. Kagan and Meranze (1957) avoided this complication in part of their work by immunizing with all-male cercariae. Following this procedure, they found no differences in

pathology or the extent of the immune response to liver worms in first and subsequent infections.

Lin and Sadun (1959) and Lichtenberg and Ritchie (1961) reported that some challenge worms reached the liver but that they were stunted and under-developed. The latter authors found such specimens for as long as 60 days. These same authors reported that no further cellular reaction was evident until after deaths of the worms, while Sadun (in Stirewalt, 1962) reported intense perivascular cellular reactions against immature schistosomula.

The disparity in size between S. mansoni or S. japonicum and T. ocellata probably accounts for some of the apparent differences in cellular reactions. Cellular accumulations against T. ocellata or its metabolic products can achieve close contact with the worms, since they are located for the most part, in sinusoids. The human schistosomes are restricted by their size to the larger vessels thus leaving reactionary cells in perivascular areas around the vessel containing the worm.

The occurrence of worms in the kidney is an indication of successful passage through lung and into the systemic circulation. In initial infections kidney contained a significant proportion of the worm burden early in the infection and was positive on days 1 - 6, 8 - 11, 14 and 21 (Fig. 39). That first challenge worms were able to evade the lung on a regular basis is revealed by the frequency with which they appeared in liver (Fig. 1) as well as kidney. In subsequent challenges, however, worms were found in kidney on only one occasion, indicating the efficiency of the lung barrier.

With one exception, there was no indication that worms of

challenge infections reached the intestinal veins. In the one case where adult(s) were seen (day 14) it is difficult to be certain whether or not they were from a previous infection. Forty-eight days had elapsed since the first challenge and over a year since the initial infection. In view of the efficacy of the immune response reported above and in light of the known longevity and egg-laying capacity of initial infection adults (Rau, 1969), it seems likely that these worms represent the initial infection of over a year earlier.

II GROWTH, MATURATION AND CONDITION OF WORMS:

As illustrated in figure 12, growth of worms was severely limited in most immune birds, in 16 of 19 birds after first challenge the parasites showed no signs of sexual development, and the gut remained in a form typical of the early schistosomulum. There were indications of damage and/or malfunction of the gut (hematin seen in parenchyma and empty ceca) and a host response to the tegument (mucoid substance and host cells stuck to worms, predominantly to "lung" worms). A similar phenomenon has been reported for adult S. mansoni retrieved from immune rhesus monkeys two days after being transferred there from normal monkeys (Mockley and Smithers, 1970). Worms from the skin were not affected in this way and fewer from liver were so affected than from lung, indicating, as revealed in sections, that this may be a function of mucus-producing cells in the bronchial network of the lung.

Lichtenberg and Ritchie (1961) summarized the effects of immune rhesus monkeys on worms of challenge infections of Schistosoma mansoni:

i) The trapping phenomenon - the proportion of worms reaching the

portal habitat being smaller than in the normal host, suggesting that the schistosomula are retained at their earlier points of migration.

ii) The stunting phenomenon - many of the worms which did arrive at the portal vessels remained immature up to the 42nd day, when ordinarily all should be fully developed.

iii) The premature extinction phenomenon - worms which did reach the portal circulation died before the 65th day.

Parts or all of these phenomena have been described by many other workers with various host-parasite combinations (e.g. Kagan, 1952; Olivier and Schneidermann, 1953; Vogel and Minning, 1953; Lin and Sadun, 1959; Magalhaes, 1959; Naimark et al., 1960; Davis et al., 1963; McMullen et al., 1967).

All of these phenomena are operative in the T. ocellata - duck system presently under consideration except, as with initial infections, these events are telescoped into a relatively short period. Whereas all challenge S. mansoni were eliminated by day 65 (Lichtenberg and Ritchie, 1961) or 97 (McMullen et al., 1967), 2 weeks was the approximate time for this to occur in ducks infected with T. ocellata.

It is of interest, of course, to know whether or not these phenomena have an immunological basis. Clegg and Smithers (1972) have shown that schistosomula of S. mansoni cultured in vitro in immune serum are stunted and die much earlier than controls grown in normal serum. Moreover, this effect can be absorbed from immune serum by adults cultivated in vitro. After inactivation, hyperimmune serum inhibits growth but does not kill schistosomula, indicating that the effect is complement dependent. The classical and only conclusive method of demonstrating an immunological basis for resistance is by

passive transfer, a subject to be dealt with presently.

In summary then, migration is slowed, with skin, lung and liver all involved in this process. There is a possibility of IgA as well as cellular components having a role in this reaction. This slowed migration coupled with the retarded development and failure to reach ovipositing sites explains Rau's (1969) findings that no egg passage occurred after challenge infections.

The similarity between these reactions and those found to occur in rhesus monkeys infected with S. mansoni are striking (Kagan, 1966). Similar effects on schistosomula and similar reactions by the host have been reported in animals immunized with attenuated cercariae (Hsu et al., 1963) and in abnormal hosts (Lichtenberg et al., 1962).

III ULTRASTRUCTURE:

Until publication of Threadgold's LM description of the tegument of Fasciola hepatica in 1963, the trematode's covering had been described as a cuticle, the exact nature of which was poorly understood (Hyman, 1951, Senft et al., 1961). Since 1963 many other trematodes have been studied, among which were schistosomes (revieved by Lee, 1966 and 1972) including one species parasitic in birds (Logachev, 1964).

With minor variation from species to species and on different parts of individual worms, the tegumental structure has proven to be essentially the same in all trematodes studied, namely a cytoplasmic syncytial epithelium connected to subjacent tegumental cytons by internuncial processes (Smyth, 1966, Smith et al., 1969).

T. ocellata is no exception, the description presented in this study being in essential agreement with descriptions of S. mansoni published by Morris and Threadgold (1968), Smith et al., (1969), Bruce et al., (1970 and 1971), and Hockley and McLaren (1973).

Particularly germane to the central theme of this study are the natures of the 3 types of granular inclusions found in the tegument of T. ocellata, namely the rod-like or discoid inclusions, the laminated vesicles, and the dense bodies. Similar inclusions have been described in S. mansoni (Morris and Threadgold, 1968; Silk et al., 1969; Smith et al., 1969; Hockley and McLaren, 1973) but exact comparisons are difficult to make.

The commonest inclusions described by these workers are the rod-like bodies which occur in all stages of S. mansoni. Smith et al. (1969) noted that these rod-like inclusions and the tegumental spines reacted the same way when treated with phosphotungstic acid, and suggested that a relationship may exist between the two. Unlike S. mansoni, which has a spiny tegument at all stages, T. ocellata loses its cercarial spines during the schistosomular phase, and in the adult spines are found only in the gynecophoric canal of the male. It may be significant then, that rod-like inclusions were common in early schistosomula but were rarely seen after day 4 and were not seen at all in adults on days 8, 9 and 10. The spatial relationship between the rod-like inclusions and the spines of T. ocellata (Fig. 24) plus their simultaneous disappearance from developing worms, supports the suggestion that a direct relationship exists between the two. Perhaps the rod-like inclusions act as "building blocks" for the protein of the spines.

The membranous or laminated vesicles are thought to aid in development of the plasma membrane during growth of the schistosomulum and to help maintain its integrity during adult life (Hockley and McLaren, 1973). Membranous bodies with granular centers similar to those described here for T. ocellata (Fig. 25) have also been seen in S. mansoni (Morris and Threadgold, 1968; Smith et al., 1969). Hockley and McLaren (1973), however, described the laminated bodies of S. mansoni as masses of tightly packed membranes with no granular center. These same workers found the tegumental membranes to be heptalaminate and attributed the divergence of their observations from the trilaminate structure seen by others, including the present writer, to better preservation of the plasma membranes following their use of uranyl acetate as a fixative.

Finally, dense bodies similar to those described in all stages of T. ocellata have been reported in S. mansoni, but only in developing worms, not in adults (Smith et al., 1969). Bruce et al., (1970) speculated that these inclusions represented secretory droplets which changed in electron opacity as they travelled from the cyton to the tegument proper. In view of the activity of the tegument in active transport (Smyth, 1966) it might be suggested that these droplets are enzymes involved in that process. However, if this were the case, one would expect them to persist in S. mansoni as they do in T. ocellata. Possibly their continued occurrence in T. ocellata is related to the speed of development of this species, most individuals having grown, matured, reproduced, and died in a shorter time than S. mansoni requires for maturation alone. The empty gut of adult T. ocellata may point to a disproportionate use of the tegumental membranes for active transport,

thus also accounting for the continued presence of the "enzymatic" secretory droplets.

Senft et al., (1961) were the first to publish electron micrographs of the gut of S. mansoni but their work was done before the perfection of many routine EM procedures and yielded little definitive information. Morris (1968), Spence and Silk (1970) and Bruce et al., (1971), on the other hand, described the fine structure of the S. mansoni gut epithelium and included excellent electron micrographs. Morris (1968) also reported that degenerating white blood cells were present but that red blood cells were absent in the lumen of the gut just as the present study has shown for T. ocellata. Morris gave evidence that the dense material in the lumen is hematin and he speculated that the lipid-like material is an intermediate of hemoglobin degradation.

"It may be speculated that extracellular digestive enzymes, perhaps produced by the oesophagus, initiate the digestion of hemoglobin. The digestive process may then be completed after uptake of the droplet by the epithelium".

A droplet apparently in an initial stage of uptake can be seen in Fig. 27 (arrow).

Bruce et al., (1971) described two distinct areas of the oesophagus separated by the degree of villosity and also described the ceca as being surrounded by a single layer of circular muscle. The musculature of the T. ocellata gut varied considerably, different sections showing none, one, or two muscle layers.

The rationale for doing ultrastructural work on T. ocellata was to make visible any damage done to the tegument by the antibodies

which are known to be made against it (Smith, 1971). That humoral antibodies can damage the tegument in an observable fashion was demonstrated by Smithers et al. (1969) in a rather artificial system wherein worms grown in mice were transferred into the hepatic portal veins of monkeys previously made immune to mouse spleen, liver, or red blood cells. The tegument underwent catastrophic degeneration and the worms died within 25 h after transfer. Ferratin-labelled rabbit anti-monkey gamma globulin demonstrated that the reaction was directed against the tegument and caused breaks in the plasma membrane followed by vacuolation and then degeneration of the underlying syncytium.

In a less artificial system, Hockley and Smithers (1970) transferred 190 pairs of 6 week-old adult worms from an initial infection in the monkey into a known hyperimmune recipient monkey. When recovered 2 days later, many parts of the tegument were normal but some areas showed degenerative changes which ranged from an increase in the density of the outer part of the tegument, to complete loss, exposing the underlying muscle fibres.

"It is probable that similar damage occurs during response to reinfection by an immune host, that is to say the target antigens affected by the immune response are located at the surface of the invading schistosomula and they will be killed following the breakdown of their tegument" (Hockley and Smithers, 1970).

It is unfortunate that technical problems associated with retrieving schistosomula from immune hosts prevented these authors from testing their hypothesis.

Electron micrographs of worms recovered from hyperimmune ducks in the present study revealed surprisingly few indications of degenerative changes in the tegument. Even at day 9 when most challenge worms had been eliminated, there were few indications of actual breaks in the superficial plasma membrane. Worms were, however, kept in a juvenile condition as indicated by the spines and discoidal bodies still present on day 9 of challenge. (Dumonde et al., 1961, have shown that histochemical techniques can reveal alterations of cells exposed to immune serum even though no morphological changes are apparent at the EM level). The high concentration of dense bodies in the teguments of challenge worms (fig. 30) may be related to the worm's defense against an immunological attack, or may simply be related to its juvenile condition.

It would appear from these results that schistosorula of I. ocellata are able to maintain themselves in a resistant host, albeit in a stunted and retarded condition, until the integrity of the tegument is lost. Once the tegument has been breached, the worms must be resolved very quickly. This view is supported by the rapid disappearance of adults from inflamed lungs in initial infections (Ellis, 1968).

IV PEROXIDASE:

The peroxidase-labelled antibody technique (Graham and Karnovsky, 1966) has proven to be a sensitive method of locating antigenic sites at either light or EM levels (Nakane and Pierce, 1966; Leduc et al., 1969). While the results of this experiment are limited, they are indicative of an antigen-antibody reaction at the surface of the

worm since electron dense granules were localized sporadically on the worm's surface. There was no indication, however, of ingestion of these complexes as suggested by Smith et al. (1969).

Hockley and McLaren (1973) have presented evidence that the superficial plasma membrane in S. mansoni is continually being renewed from within so that membranes damaged by an antigen-antibody reaction would be quickly replaced. Thus any antigen-antibody complex at the surface would likely be transitory. This phenomenon may explain Smith's (1971) negative FA results with live schistosomula of T. ocellata tested after recovery from immune hosts. The high numbers of granular inclusions in the tegument of worms from immune hosts (fig. 30) may also be related to continual replacement of the outer plasma membrane.

As indicated earlier, the amount of gamma globulin available was sufficient to perform this test only once and it is regrettable that not enough worms were recovered from the immune ducks to make these results more meaningful. More extensive experiments of this kind could have identified the locations of antigenic sites precisely by applying labelled antibody to sectioned material (Kawari and Nakane, 1970). However, in view of the fact that there is no necessary relationship between given specific antibodies and protective immunity (Jachowski et al., 1963; Maddison et al., 1970), it was decided not to repeat this experiment. Smith's (1971) study plus the limited results of this one experiment were enough to make attempts at passive transfer the next logical step in the present work.

It was noted that mitochondria were stained selectively in all worms treated for peroxidase (Graham and Karnovsky, 1966). This is

indicative of an endogenous peroxidase, probably involved in respiration (Threadgold et al., 1968). In one of two worms from the immune duck (fig. 33) there appeared to be an increase in the number and a change in the complexity of the mitochondria. This may be related to a change in metabolism as a result of the immune response directed at the worm (Luft et al., 1962).

V BENZPYRENE TECHNIQUE - THE INTEGRITY OF PLASMA MEMBRANES:

The use of 3:4-benzpyrene as a lipid-soluble fluorochrome was described and extensively analyzed by Berg (1951). Bitensky (1967) outlined methods for its use on live and fixed tissues and described its use in experiments on the cytotoxic effect of antibody and complement on EL_4 mouse ascites cells. Suspensions of untreated cells showed only general fluorescence in the cytoplasm when studied with benzpyrene - indicating that the lipids of the membranes were well masked. In contrast, suspensions of cells treated with antibody and complement showed strong staining in benzpyrene (Bitensky, 1963).

After using the FA technique, Moore (1967) concluded that the cuticular (tegumental) complex and the lining of the alimentary canal were the major antigenic sites in adult S. mansoni and Smith (1971) in her FA study of T. ocellata came to a similar conclusion. It is interesting to note that the usual sites of membrane damage revealed by benzpyrene in the present study included these two areas. This, however, does not establish a cause and effect relationship between the antibodies revealed by the FA technique and the damage to cell membranes revealed by the benzpyrene technique.

In view of the tegumental destruction wrought in S. mansoni by

immune forces (Smithers and Terry, 1969a) and knowing that an equally potent immunity is developed against T. ocellata, the present writer had expected to see marked derangement of the teguments of worms recovered from immune birds. The fact that such worms displayed almost normal teguments, coupled with the clear demonstration by benzpyrene that scattered foci of membrane damage exist in worms taken from immune hosts, implies that continual replacement of tegumental membrane as described for S. mansoni (Hockley and McLaren, 1973) probably occurs in T. ocellata as well. It would seem that these regenerative forces are able to contend with immune lesions for a time but that at some point defenses collapse and rapid destruction of the parasite ensues.

VI IMMUNOSUPPRESSION:

To identify more specifically the agency responsible for resistance in ducks, an attempt was made to abolish the immune response. Nine ducklings were exposed when 2 or 3 days old to graded doses of gamma irradiation (Co^{60}) and subsequently exposed to cercariae. During patency, miracidial counts were made to facilitate comparison of infections in irradiated birds with the patterns established by Rau (1969) for normal ducklings and ducks. Hosts with reduced immunological competence are known to show patterns of parasite egg passage which are elevated and prolonged (Rau, 1969; Maddison et al., 1971). Therefore, in order that comparisons might be made, the daily miracidial counts were represented as percentages, the total number obtained from each duck being 100% for that particular host. The mean percent after peak in the experimental birds in this study was not significantly higher than that

in ducks (p 0.4) or ducklings (p 0.2) in Rau's (1969) study (Appendix IV).

After homologous challenge, 8 of the 9 birds developed no infection and the ninth developed only a low grade parasitemia (Table VIII). These results, being indistinguishable from those derived after challenge of normal birds (Rau, 1969), indicate that complete resistance to I. ocellata was acquired by the irradiated ducklings.

Since the attempt at immunosuppression failed to alter the bird's capacity to develop resistance to reinfection, one wonders whether immunosuppression had, in fact, been achieved. No serological technique was used to monitor the immunological competence of the ducks, but clearly, the exposure to 800 rads was about the maximum tolerable dose, the 2 ducklings exposed to 900 rads dying within 12 h. These data confirm Abraham's report (1972) that the $LD_{50}(30)$ for 4 month-old mallards was 704 rads, with 55 % of all deaths occurring within 10 d. Further, at least 4 birds in the present study were exposed to cercariae at day 9 or 10 when leucocyte levels were shown to be low.

On the other hand, it may be that abolition of the immune response in birds is not possible using irradiation alone. Cooper et al., (1966) produced agammaglobulinemia and complete failure of antibody production in only 40 % of the domestic fowl which they had surgically bursectomized at hatching and then given total body irradiation. Rose and Orlans (1968) reported even less success after similar treatment. It would appear that the best method of inducing an immunosuppressed state is with a combination of hormonal in ovo bursectomy and repeated administration of cyclophosphamide during the first few days after hatching

(Weber, 1972). However, since attempts to effect chemical bursectomy in ducklings have failed (Bourns, unpublished) and the histological changes which one might expect in the bursa of Fabricius following irradiation (Montour, 1971) have not materialized (Ellis, unpublished), it becomes apparent that methods used in the study of domestic fowl may not be applicable to experiments with ducks and that much basic information relating to the tolerances and responses of anatid birds is presently unavailable.

Mammals are known to be more radiosensitive than birds (Casarette, 1968) so it is not surprising that immunity to Trypanosoma duttoni was suppressed for up to 15 days after mice had been irradiated and that immunity to trypanosomes was suppressed for up to 23 days in rats (Kaiman, 1944). Partial suppression, as evidenced by enhanced infections, has been reported following irradiation of rats with Hymenolepis nana (Coleman et al., 1967), rats with Hyditigera taeniaformis (Myant et al., 1959), and mice with Trichinella spiralis (Stoner and Hale, 1952; Varinsky, 1962).

On the other hand, irradiation of rats (550R) at day -2 or +18 failed to alter the normal pattern of S. mansoni infection wherein parasitemia is terminated 4 - 8 weeks after exposure to cercariae (Maddison et al., 1970). Thoracic duct drainage, ALG, and a combination of ALG and Imuran reduced circulating antibody as determined by the IdA, CF, sF, CnR, and PCA tests, but were equally ineffectual in changing the duration of the parasitemia, thus confirming the finding of Jachowski et al. (1963) that the serological and immunological properties of a host are not necessarily comparable.

Of greater interest in the present context, because of other similarities between the two host-parasite models, are the reports by Waddison et al. (1971) that S. mansoni egg output from 2 nonimmune irradiated rhesus monkeys was indistinguishable from that produced by non-irradiated controls, and by Waddison and Kagan (1970) that "in no case was a second dose of infecting cercariae capable of developing into mature egg-passing adults in previously exposed, immunosuppressed monkeys."

Thus, failure has followed each attempt to use immunosuppression as a means of establishing unequivocally that acquired resistance to schistosomiasis mansoni in the rhesus monkey and trichobilharziasis ocellata in ducks is an immunological phenomenon.

VII PASSIVE AND ADOPTIVE TRANSFER:

There is no evidence in any helminth infection that cell-mediated immune mechanisms are the sole mediators of immunity (Soulsby, 1972) and the only two reported attempts to transfer immunity to schistosomes via lymphoid cells were unsuccessful. However, one involved a highly susceptible host, the mouse, which develops only marginal resistance to reinfection (Hunter et al., 1967) and the other an innately resistant host, the rat, which will not support a primary infection to egg-laying maturity and in which no immunological basis has been established for the innate resistance which exists (Waddison et al., 1970; Smithers, 1972). It is likely that other unsuccessful efforts have been left unreported.

In the one mammalian system where good resistance develops after a normal primary infection (S. mansoni in rhesus monkeys; Smithers

and Terry, 1965) a more fundamental problem has prevented experiments on adoptive transfer - the lack of isogenic strains of monkeys in which to do the work.

While isogenous strains of ducks do not exist either, the telescoped growth events of I. ocellata, plus the continued possibility of ducks tolerating foreign protein after hatching (Hasek et al., 1961) permitted this hypothesis to be tested in the present work, since transferred cells need remain viable for only a few days compared to several weeks in the S. mansoni - rhesus model. Allogeneic cell transfers have been successful in transferring immunity to Haemonchus contortus in sheep (Scott et al., 1971) but the danger of a graft versus host reaction exists when the recipients are young and immunologically incompetent (Terasaki, 1959). Spleens of ducks having received cells were only slightly heavier than the untreated birds at the time of sacrifice, however, indicating that this complication had not become a serious problem in the present work. My birds probably did not live long enough to develop overt signs of the wasting disease or runt disease described by Terasaki (1959) in chick embryos which had been injected with adult lymphoid cells.

There was no evidence in the present work that immune spleen cells with or without serum, or diverticulum cells, had a deleterious effect on schistosomula of I. ocellata when transferred to young ducklings (Tables IX to XI). In fact in one case, (Table X) the worms from the untreated duck were significantly shorter than the worms from either the hyperimmune cells-treated or the normal cells-treated duck, an anomalous result perhaps due to physiological differences between ducklings.

Prevailing opinion seems to favor humoral antibodies as the main source of immunity to schistosomes (Smithers et al., 1969a; Soulsby, 1972) although a cellular mechanism is known to be concerned in the formation of granulomata around tissue-trapped eggs (Warren et al., 1967).

In 3 of 6 groups involving transfer of cells and serum and in 2 of 3 groups involving transfer of serum alone, the worms from normal serum-treated ducks were longer than the worms from either the untreated or the hyperimmune serum-treated birds and the difference was statistically significant in every case but one (Tables XI and XII). This phenomenon may be due to normal adult serum providing an enhanced nutrient environment when compared to untreated ducklings, it being known that I. ocellata grows at a faster rate in adults than in ducklings (Ellis, 1966).

Almost certainly for different reasons, experiments involving immunization by vaccination with extracts of adults etc. have also produced apparently anomalous results. Ritchie, Garson and Erickson (1962) for instance, immunized mice, in sequence, with cercarial antigen, worm antigen and egg antigen, simulating the natural order of events. Recovered and measured 7 weeks after exposure, the worms from 4 of 5 experimental groups were significantly longer than those from saline controls. Maddison et al., (1971) reported similar results after sensitizing rhesus monkeys with extracts of adult S. mansoni.

Almost all attempts to transfer immunity passively in serum or gamma globulin have been unsuccessful (See summaries by Stirewalt, 1963; Kagan, 1966; and Smithers and Terry, 1969a). Kawamura (1932)

gave qualitative evidence of having passively transferred immunity to S. japonicum in dogs and rabbits, but this could not be substantiated by Vogel and Minning (1953). "Successful" passive transfer has meant slight reductions in the numbers of maturing worms and even this degree of success has been achieved only after the use of heterologous serum (rabbit to mouse, Sadun and Lin, 1959; and monkey to rats, Bruce and Sadun, 1964).

Since Smithers and Terry's review (1969a) there have been several more unsuccessful attempts at passive transfer (Maddison et al., 1971; Tewari and Biswas, 1972; Cook et al., 1972; Warren et al., 1972). The latter two reports are especially interesting since they involved attempts to prevent and cure bilharziasis in children in a field situation. Relatively large amounts of gamma globulin were injected (108 mg/Kg).

Most studies, including the present one, have involved the use of moderate amounts of whole serum (e.g. Stirewalt and Evans, 1953, 2.1 mls in mice; Sadun and Lin, 1959, 2 mls in mice; Levine and Kagan, 1960, 5 mls in mice; Weinmann and Hunter, 1961, 10 mls in mice; Maddison et al., 1971, 1 ml/Kg body weight in rats). Larger volumes of serum have been used in a few studies. Weinmann (1960) reported the lack of protection in neonatal mice born of hyperinfected mothers. Meisenhelder et al., (1960) replaced approximately 3/4 of the total blood volume of a normal rhesus monkey by blood from the monkey with a 30 month old infection. This failed to protect against a challenging infection. Finally, Hunter et al. (1967) reported that even parabiosis of infected and non-infected mice failed to protect the uninfected partner. Despite these failures,

a renewed effort along the same lines was warranted in the duck-schistosome model because: i) Complete resistance to challenge has been shown to follow initial exposure (Rau, 1969). ii) Early parts of the present work indicated that immune serum might be having some effect (shortest worms from ducks receiving hyperimmune serum, although this difference was not often significant). iii) A report by Clegg and Smithers (1972) of a lethal antibody in serum of infected rhesus monkeys which was effective only at high concentrations (e.g. LD 50 (4) at titres of 1/8 - 1/64). These findings proved to be similar to Howell's results (1971, unpublished) with cultured T. ocellata.

Ducks involved in these experiments received 3 to 4 times their own fluid content in immune serum. No other passive transfer experiments with schistosomes have involved as much serum over such a relatively long time in a host-parasite system where the donors are known to be completely resistant to challenge. In the first experiment, where worms were recovered and measured after 4 days in their respective hosts (Table XIII), the differences between the worms were consistent and immediately obvious upon recovery. Statistical analysis served to confirm the initial impression that compared to those from either normal serum-injected ducklings or saline-injected ducklings, worms from hyperimmune serum-injected hosts were markedly stunted in growth (Table XIII).

The next logical step was to repeat the experiment letting the infection proceed to patency to assess whether or not the stunted worms seen after exposure were able to mature into egg-laying adults. An absolute resistance to challenge was not achieved using this criterion but there were distinct differences between the three groups as outlined

in figure 38 and Table XIV. Patency first occurred on day 11 or 12 in controls but not until day 16 in experimentals. All control birds became patent whereas 1 of the experimentals shed no eggs and another only 1 egg throughout the observation period.

The experiment was hampered by two related problems; missing data for 3 - 5 days in 8 of 9 controls, this being impossible to estimate afterwards because of the erratic individual egg-laying curves (See appendix IV) which resulted from the other problem, very light infections. The difference between the number of eggs recovered from the "saline" control group and the experimental group is statistically significant in any case. However, statistical significance between the numbers of eggs laid by the normal serum control and the experimental group is denied by the lack of data. There can be little doubt of the biological significance between these two groups, however, especially in the light of the stunted appearance of worms at day 4 in the earlier experiment involving massive amounts of serum. A lack of sufficient material (snails shedding cercariae of I. ocellata) has prevented a repetition of this experiment up to the present time. It would be interesting and rewarding to repeat it using adults as well as ducklings and including immunocompetent cells as well.

Smithers (1972) said that "the role of humoral antibodies in schistosome immunity can be established beyond doubt only by demonstrating the passive transfer of immunity with serum or immunoglobulin --- but this has not yet been satisfactorily achieved."

It seems safe to conclude from the present work that there is definitely a deleterious effect conveyed by passive transfer of immune serum if large enough volumes are used - an indication of low titres of

the stunting and killing antibody. however, this effect was not absolute, some worms were able to survive and, though delayed, reach sexual maturity and pass viable eggs.

It is apparent also that the host-parasite relationship is extremely complex and probably no one part of the immunological armament of the host is solely responsible for protection. This is true for other host-parasite systems as well as the one presently under consideration (e.g. malaria in mice, Criswell et al., 1971; and Trichostrongylus in guinea pigs, Connan, 1972). Homocytotropic antibodies, for instance, are a common concomitant of helminth infections including schistosomiasis (Williams et al., 1972). Even in mammals this is a little understood phenomenon "--- much additional evidence is required before the various pieces of the jig-saw puzzle can be made to fit together into a clear, coherent picture ---" (Sadun, 1972). This is an almost completely virgin area in birds and could be a fruitful research topic.

The role of IgA is of interest, especially since the schistosomula of T. ocellata challenge infections are apparently trapped in the parabronchi and secondary bronchi of lungs where IgA might be expected in high concentrations (Newington et al., 1964). "If IgA antibody at the mucosal surface is in part responsible for maintaining an immune state in a resistant host, this supposition --- may explain why relatively large doses of hyperimmune serum are required to transfer immunity passively to Nippostrongylus brasiliensis in the rat" (Nagan, 1970). This same speculation would seem to apply to T. ocellata infections in ducks and warrants further basic study.

Finally, immunocompetent and pharmocologically-active cells,

which have been shown to be important in immunity of rats to Nippostrongylus (Hoparth-Scott and Bingley, 1971) no doubt play a role in immunity to schistosomes as well. Pharmacologically-active cells were seen in the present study, at the EM level, to be in contact with worms and in some cases to have released their granular contents on or near the surfaces of the worms. Sections of skin, lung, and liver all revealed an intense cellular reaction to challenge worms and worms retrieved from immune hosts had cells attached to them. The lack of positive results after transfer of spleen cells certainly does not eliminate their importance in the overall immunity of the host. Perhaps immune serum was not present in optimum quantities and phenomena like immune adherence were not operative. Lewert (1970) suggests that the actual death and elimination of all stages of the schistosomes is due to invading cells, although it may be mediated by humoral antibody. He also cites unpublished data (Lewert and Cahill) to support the hypothesis that immobilization allows destruction of the worms: "Shortly after induction of hibernation by hypothermia of the host, the immobilized adults are found in the liver where they are invaded by leucocytes and rapidly destroyed. Within a very short time after the parasites are immobilized at 5° C *in vivo* leucocyte sheaths and accumulations are found that appear to be identical to those described by Newsome (1962) *in vitro*."

Figure 1. Distribution of T. ocellata in ducks (1st challenge)

DISTRIBUTION OF T. OCELLATA IN DUCKS (1ST CHALLENGE)

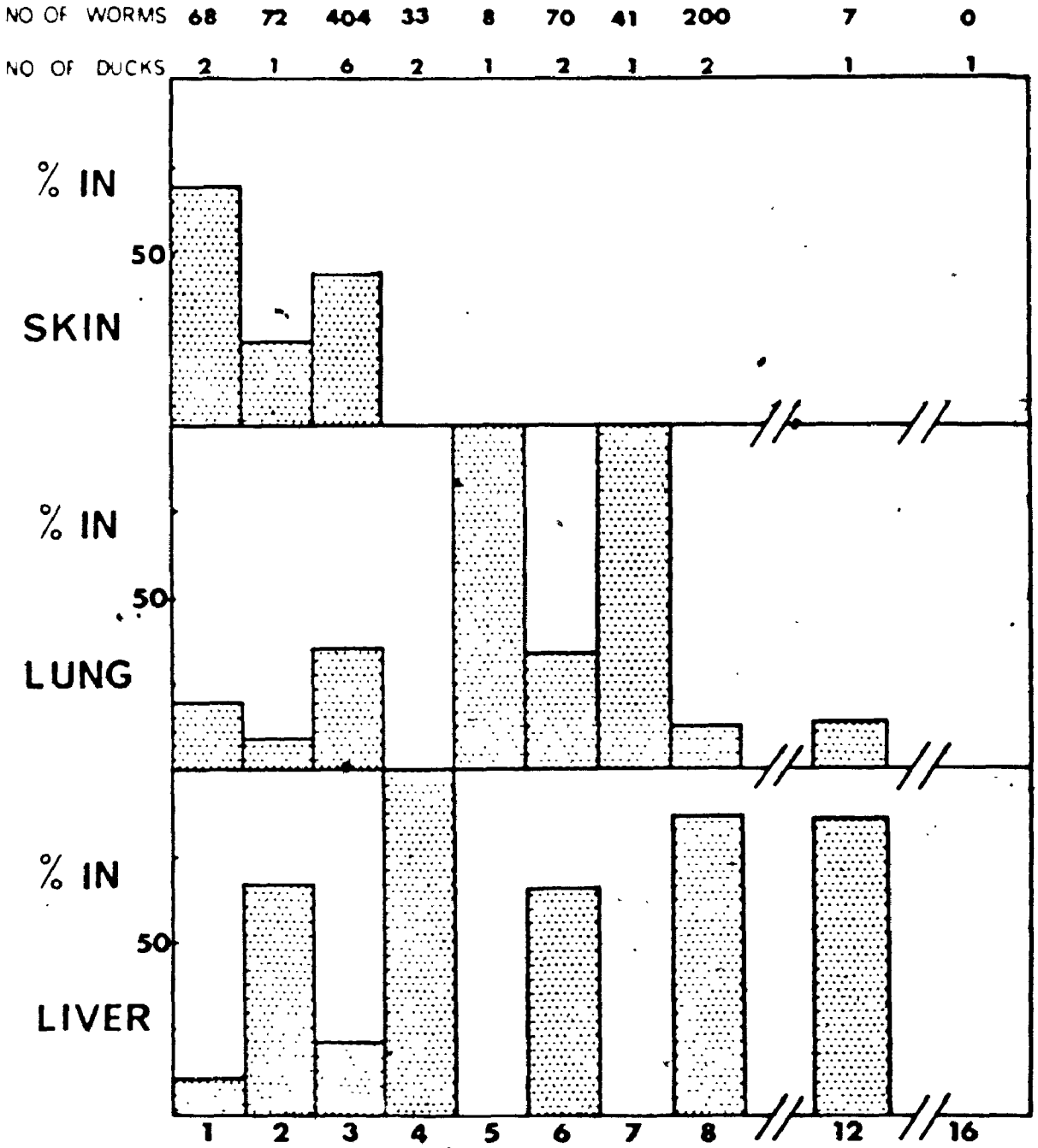


Figure 2. Section of skin 3 days after first challenge. Note cercarial body (s) in stratum corneum (SC) and cellular infiltration and debris between the stratum corneum and the stratum germinativum. X280.

Figure 3. Section of skin 5 days after second challenge. Note the separation of the layers of the stratum corneum and the intense inflammatory reaction in the dermis (d) and hypodermis (h). a - artery X280.

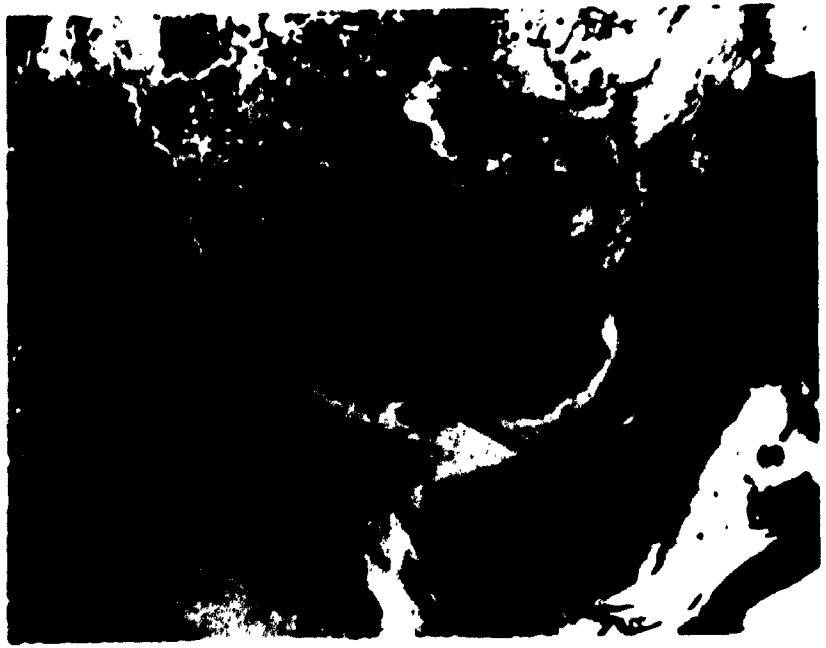
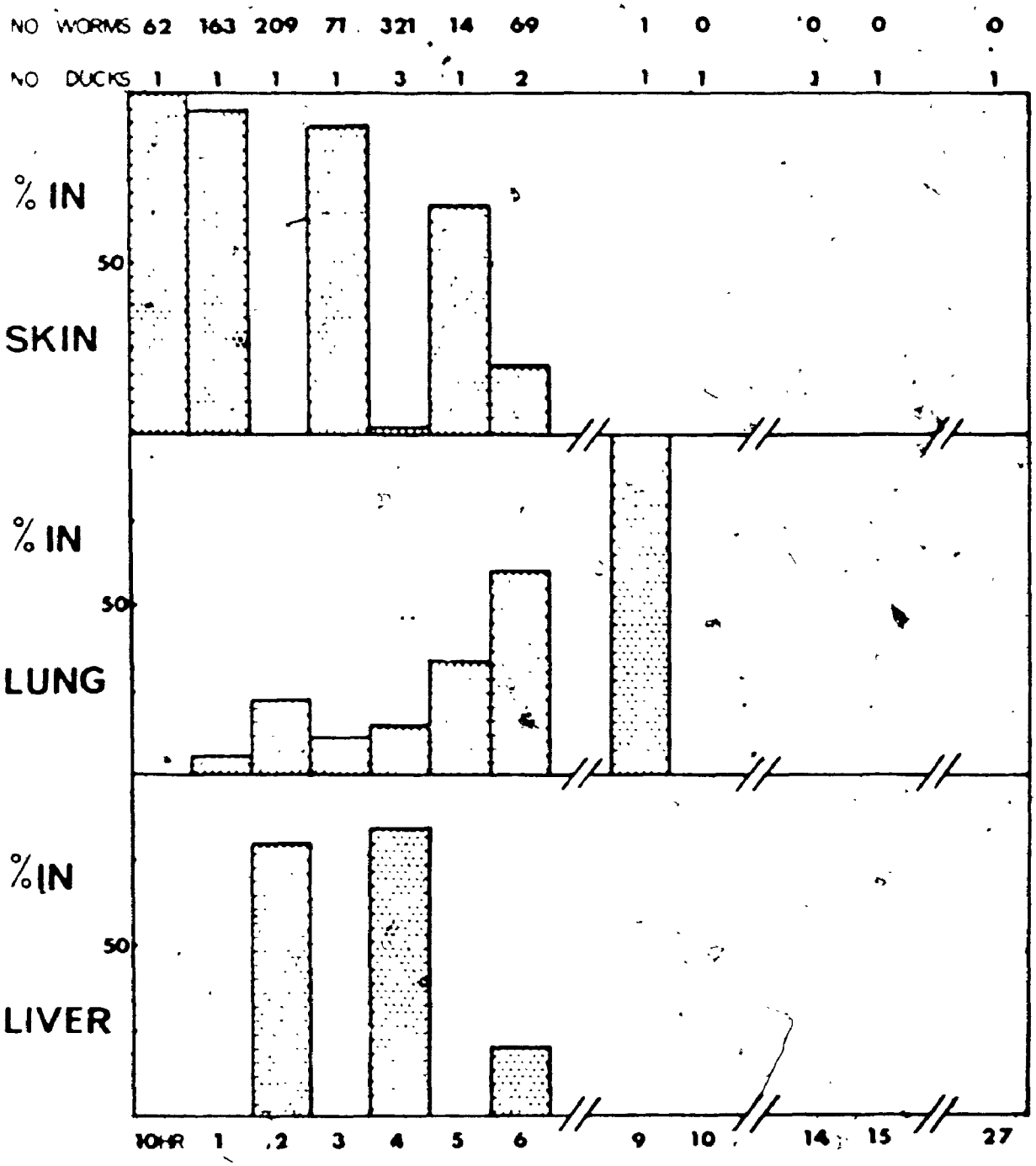


Figure 4. Distribution of *I. ocellata* in ducks (2nd challenge).

DISTRIBUTION OF T. OCELLATA IN DUCKS (2ND CHALLENGE)



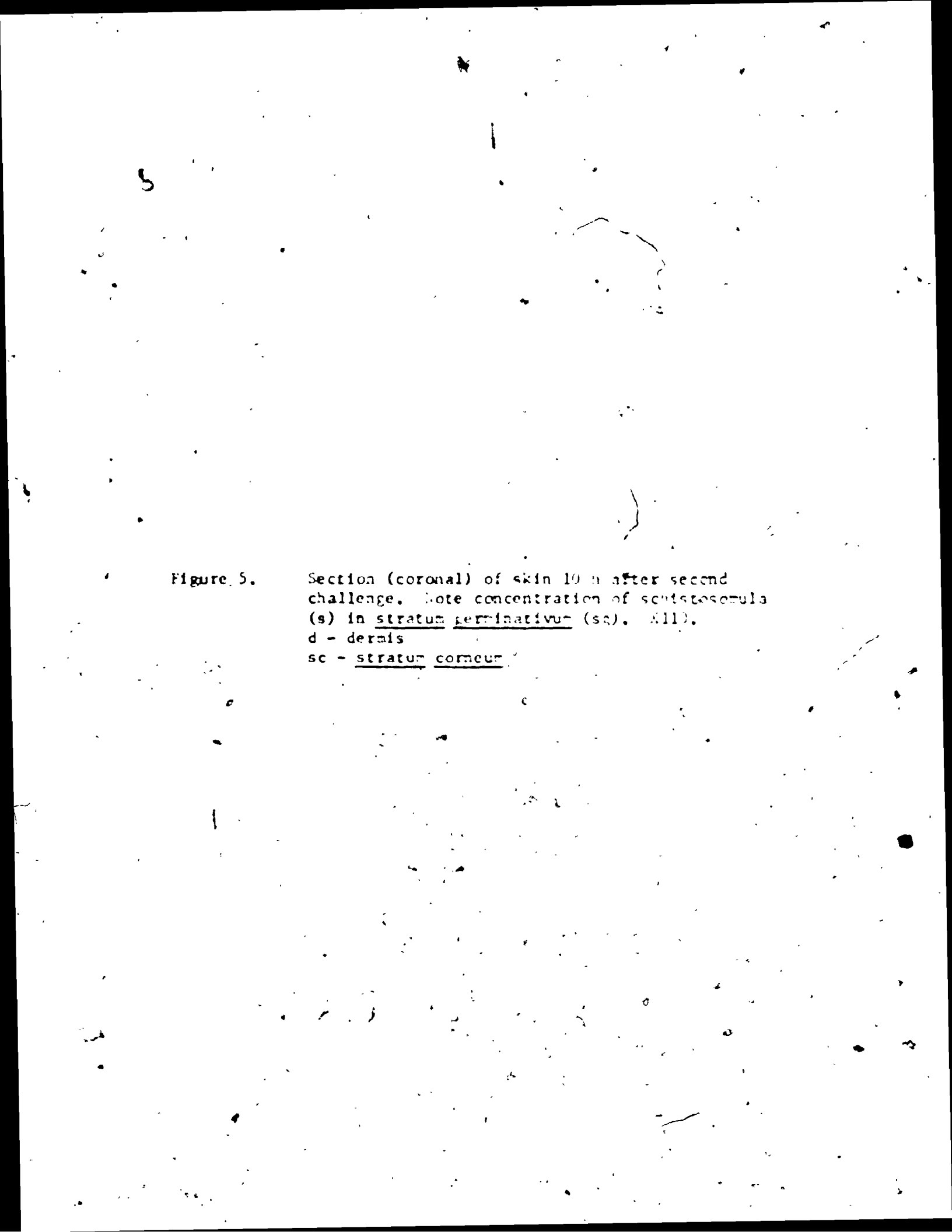


Figure 5. Section (coronal) of skin 10 h after second challenge. Note concentration of scutotesemula (s) in stratum germinativum (sg). X11).
d - dermis
sc - stratum corneum

2 2

OF/DE

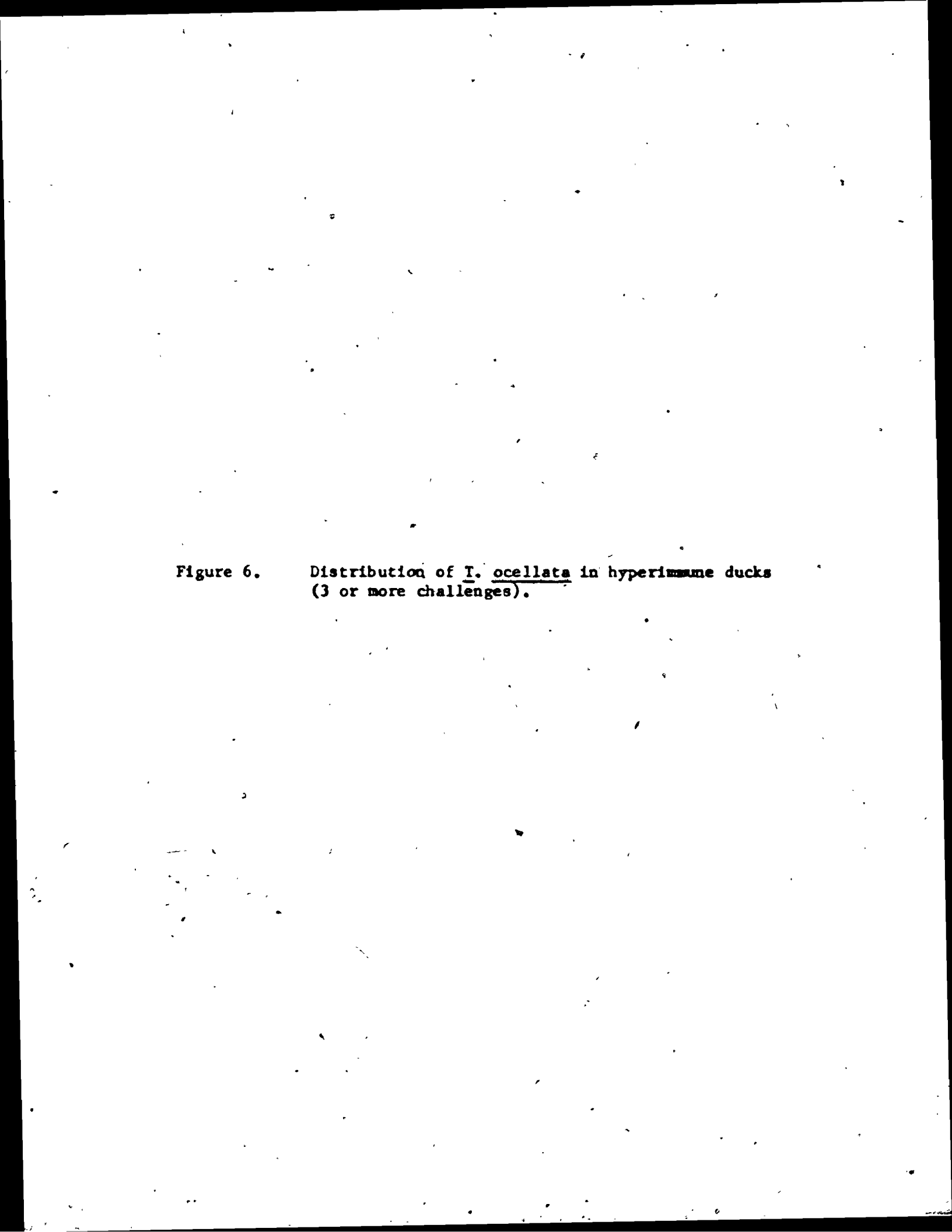


Figure 6. Distribution of *T. ocellata* in hyperimmune ducks (3 or more challenges).

DISTRIBUTION OF T. OCELLATA IN HYPERIMMUNE DUCKS (3 OR MORE CHALLENGES)

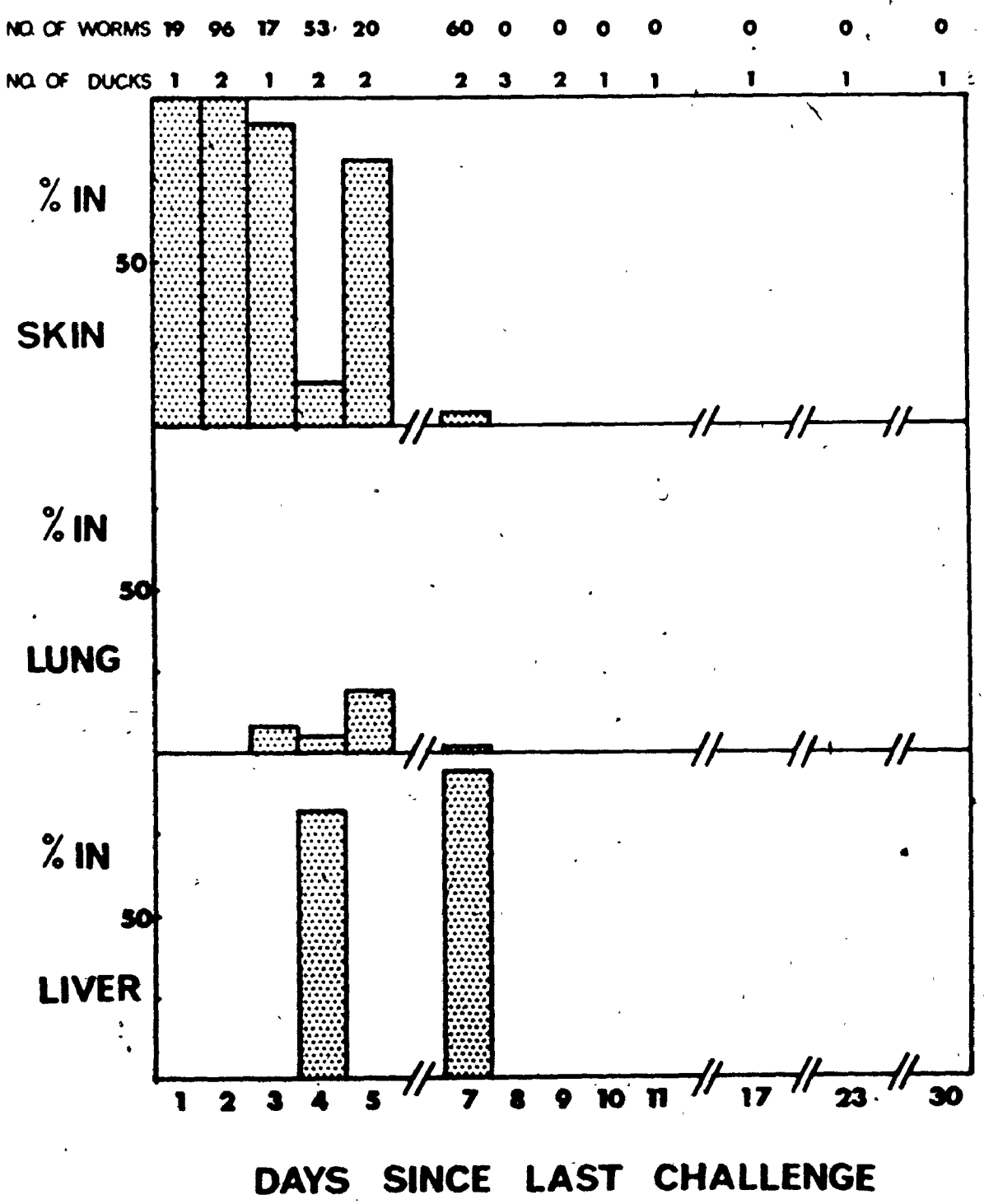


Figure 7. Section of lung 2 days after first challenge. The schistosomulum (s) is almost indistinguishable from lung tissue. X280.
g - gut of schistosomulum

Figure 8. Section of lung 3 days after first challenge. Note schistosomulum in lumen of secondary bronchus surrounded by host cells and mucoid material (m) fromgoblet cells. X280.
eg - empty gut of schistosomulum

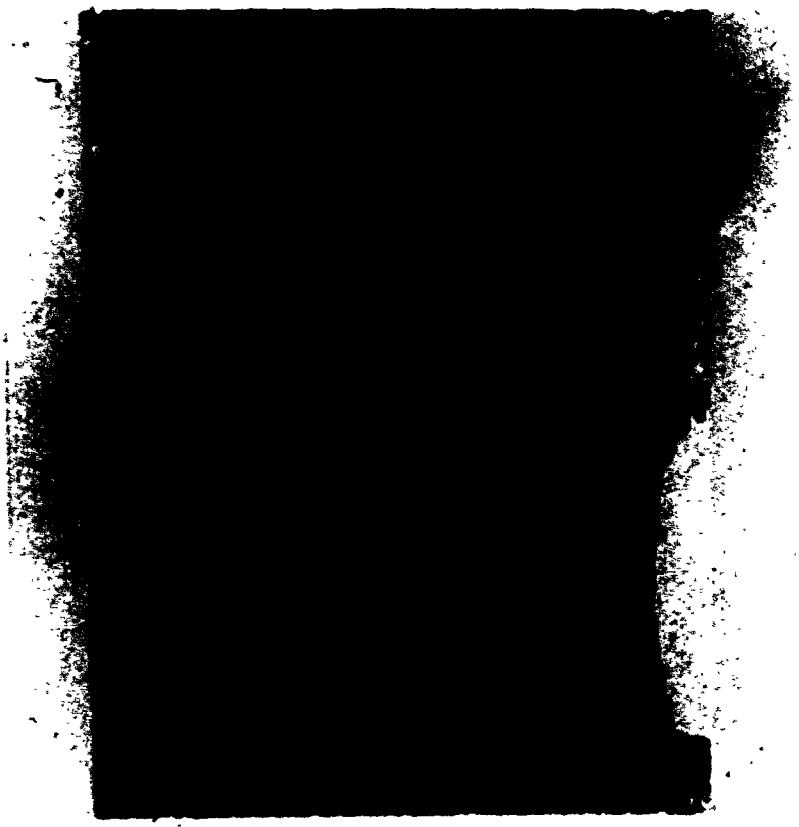


Figure 9 a Section of lung 2 days after second challenge. Schistosomulum (s) completely surrounded by host tissue. X720.
p - parabronchus

Figure 9 b Section of lung 2 days after second challenge. Areas of intimate contact and apparent adhesion are indicated by arrows. X720.
p - parabronchus

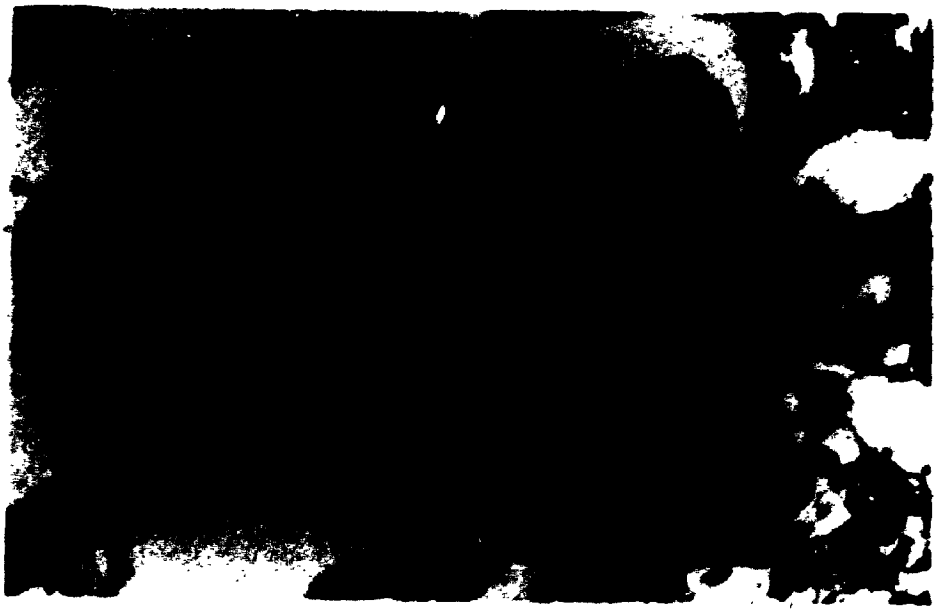


Figure 10. Section of lung 6 days after second challenge. Note schistosomulum (s), still juvenile in shape, surrounded by cells and mucoid material (m) apparently originating from cells in the epithelium (e) of the secondary bronchus. X280.

Figure 11. Section of liver 3 days after first challenge. Note schistosomula (s) in focus of lymphoid cells (lph). X280.
eg - empty gut of schistosomulum

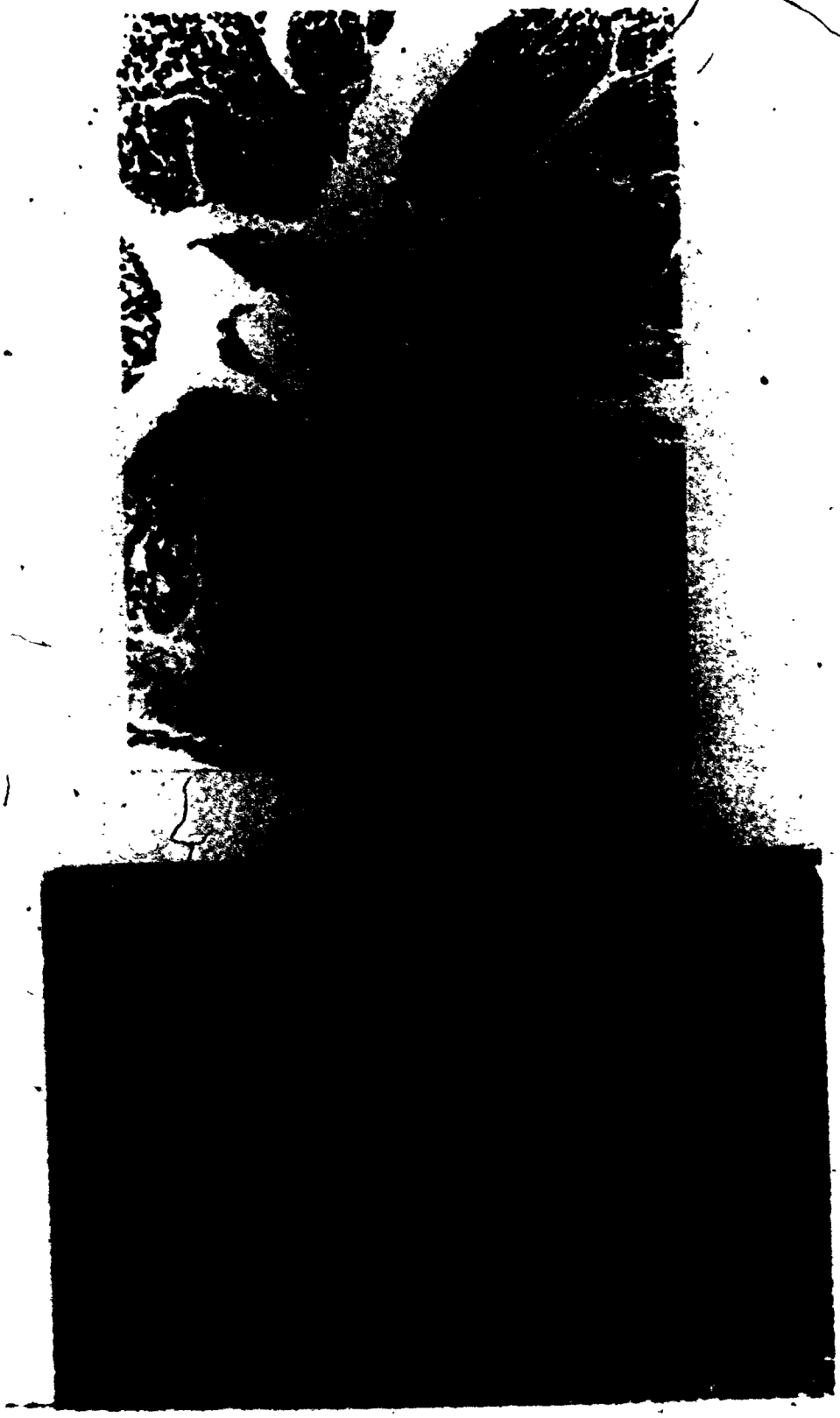


Figure 12. Mean lengths of T. ocellata recovered from ducks after initial and challenge exposures (vertical lines indicate standard deviations).

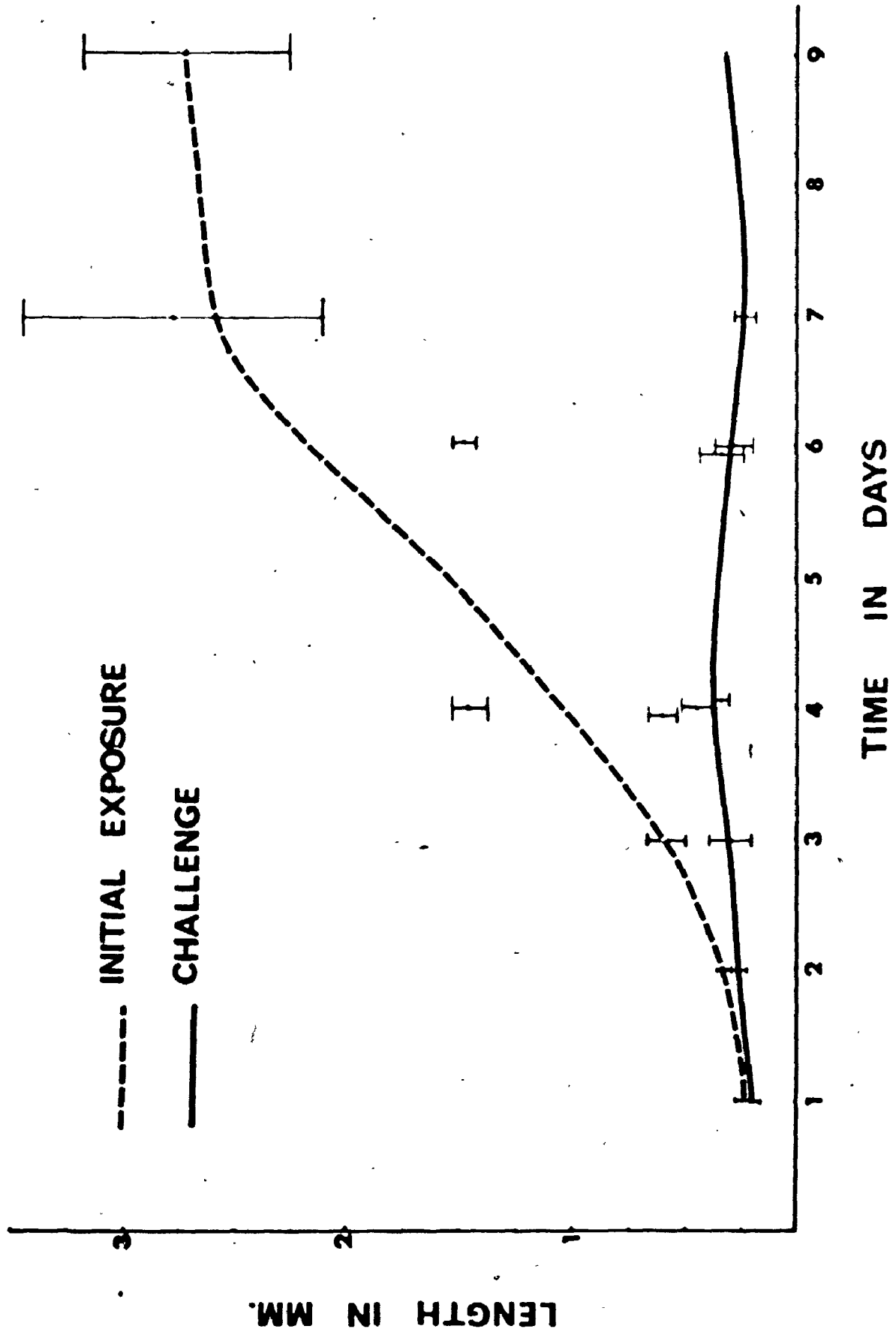

INITIAL EXPOSURE
CHALLENGE
LENGTH IN MM.
TIME IN DAYS
1
2
3
4
5
6
7
8
9

Figure 13. Day 7 worms (T. ocellata) recovered from initial (inl) and challenge (cnl) infections in ducks. X45.

Figure 14. Day 7 challenge worm. X720.
es - eye spot

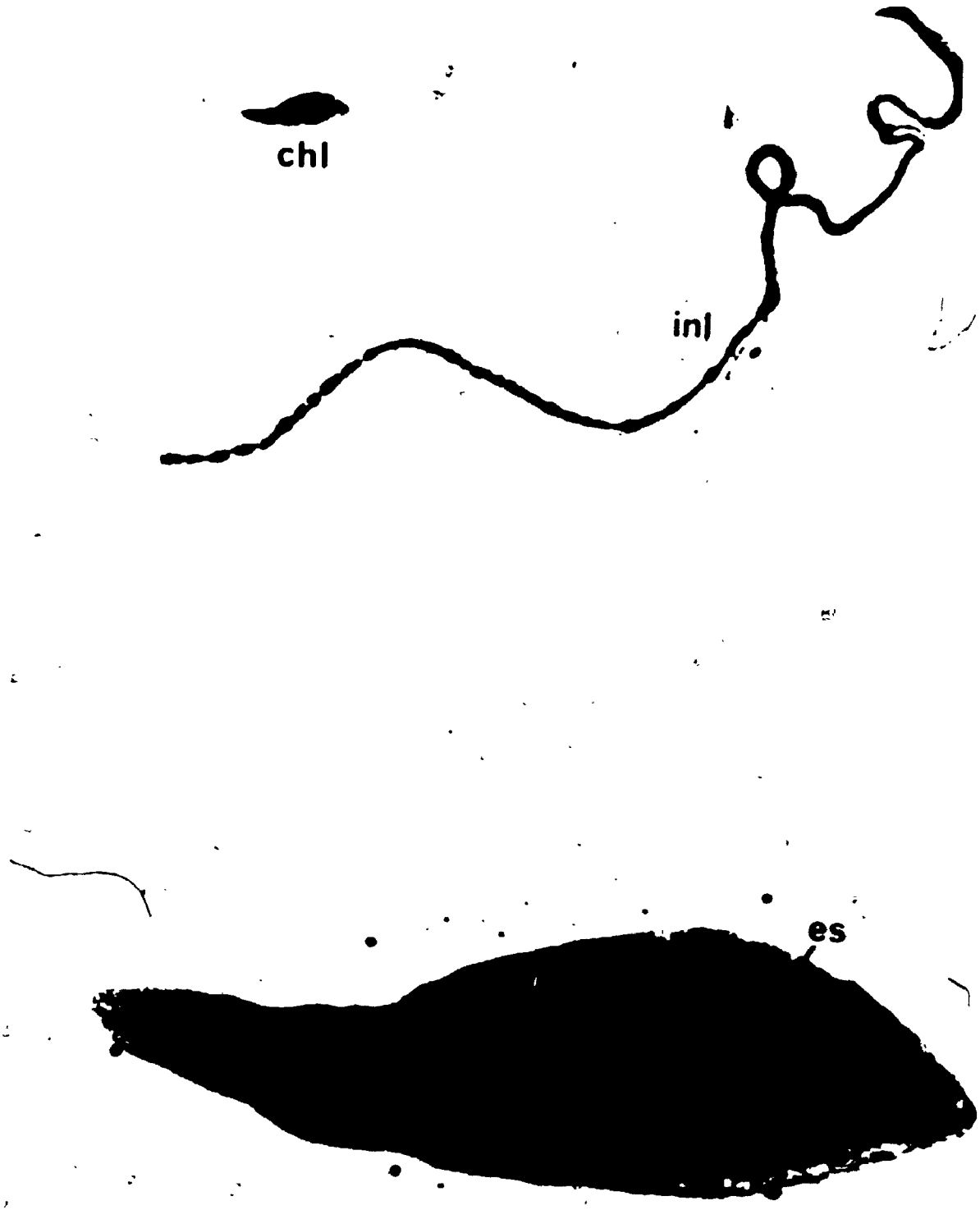

chl
inl
es

Figure 15. Schistosomulum 6 days after first challenge showing undigested cells (c) in lumen of gut (g). X1650.

Figure 16. Schistosomulum 6 days after first challenge. Surface view showing the spiny nature of the tegument. X1320.
s - spines

Figure 17. Schistosomulum 6 days after first challenge showing undifferentiated genital primordium (gp). X1320.
vs - ventral sucker

Figure 18. Schistosomulum 6 days after first challenge showing cercaria-like head organ (ho). X1320.


DP

Figure 19. Schistosomulum 3 days after first challenge showing host cells (c) and debris attached. X720.
es - eye spot

Figure 20. Electron micrograph of tegument of 2 day schistosomulum showing the trilaminate nature of the plasma membrane (tl) and an indication of a more complex, pentalaminate structure (pl). X68000.
f - fibrous layer
m - mitochondrion


ip

Figure 22. Electron micrograph of tegument of *T. ocellata* 3 days after first challenge. Note the rough endoplasmic reticulum and Golgi apparatus (G) in the nuclear portion of the tegument and the dense bodies (db) and rod-like inclusions (r) in the distal part. X30000.
f - fibrous layer
ilm - inner longitudinal muscle
lv - laminated vesicle
m - mitochondrion
n - nucleus
ocm - outer circular muscle
s - spine

Figure 23. Electron micrograph of tegument of T. ocellata 2 days after initial infection showing an internuncial process (ip) or protoplasmic extension containing microtubules (mt) and granular inclusions apparently in transit to the distal part of the tegument. X28000.

bm - basement membrane
f - fibrous layer
ilm - inner longitudinal muscle
m - mitochondrion
mt - microtubule
n - nucleus
ocm - outer circular muscle
s - spine


ip

Figure 24. Electron micrograph of part of tegument of T. ocellata containing a spine (day 9 challenge). Note crystaline structure of spine. Arrows indicate rod-like inclusions apparently lining up parallel to longitudinal axis of spine. X54000.
db - dense bodies
lv - laminated vesicle
r - rod-like inclusions
s - spine

Figure 25. Electron micrograph of tegument of *T. ocellata* (day 4 initial exposure) showing a laminated vesicle (lv) in detail. Note the granular contents. X75000.

Figure 26 a Electron micrograph of oesophagus of T. ocellata (longitudinal section). X19000.
cm - circular muscle
db - dense body
lu - lumen
lv - laminated vesicle
m - mitochondrion
n - nucleus

Figure 26 b Electron micrograph of oesophagus of T. ocellata (cross-section). X7700.
cm - circular muscle
lm - longitudinal muscle
n - nucleus

Figure 27. Electron micrograph of gut of T. ocellata. X15000. Arrow indicates possible uptake of degraded haemoglobin across the epithelial plasma membrane.
bm - basement membrane
in - invagination of bm
leu - leucocyte
m - mitochondrion
rer - rough endoplasmic reticulum

Figure 28. Electron micrograph of gut of T. ocellata (day 3) showing sheet-like lamellae (lam) on surface of epithelium. X7700. Insert (day 9) shows tri-laminate nature (tl) of the membrane. X54000.

Figure 29. Electron micrograph of tegument (day 3, first challenge) of T. ocellata. Note densification (ds) of tegumental plasma membrane. X25000.
db - dense body
lv - laminated body
n - nucleus
ocm - outer circular muscle

Figure 30. Electron micrograph of tegument of day 9 first challenge schistosomulum. Note concentration of granules in superficial part of tegument (dense-bodies and rod-like inclusions). One cell appears to be dying (dis c). X28500.
db - dense body
gly - glycogen
m - mitochondrion
n - nucleus
r - rod-like inclusions

Figure 31. Electron micrograph of tegument of T. ocellata (day 9 first challenge). Arrow indicates break in superficial plasma membrane. Note also the densification (ds) of this membrane in other parts of the section. X39000.
s - spine

Figure 32. Ultrastructure of T. ocellata's tegument showing the perinuclear cytoplasm joined to the external or distal cytoplasm by internuncial processes.
db - dense body
fl - fibrous layer
ilm - inner longitudinal muscle
lb - laminated body
m - mitochondrion
mt - microtubule
n - nucleus
ocm - outer circular muscle

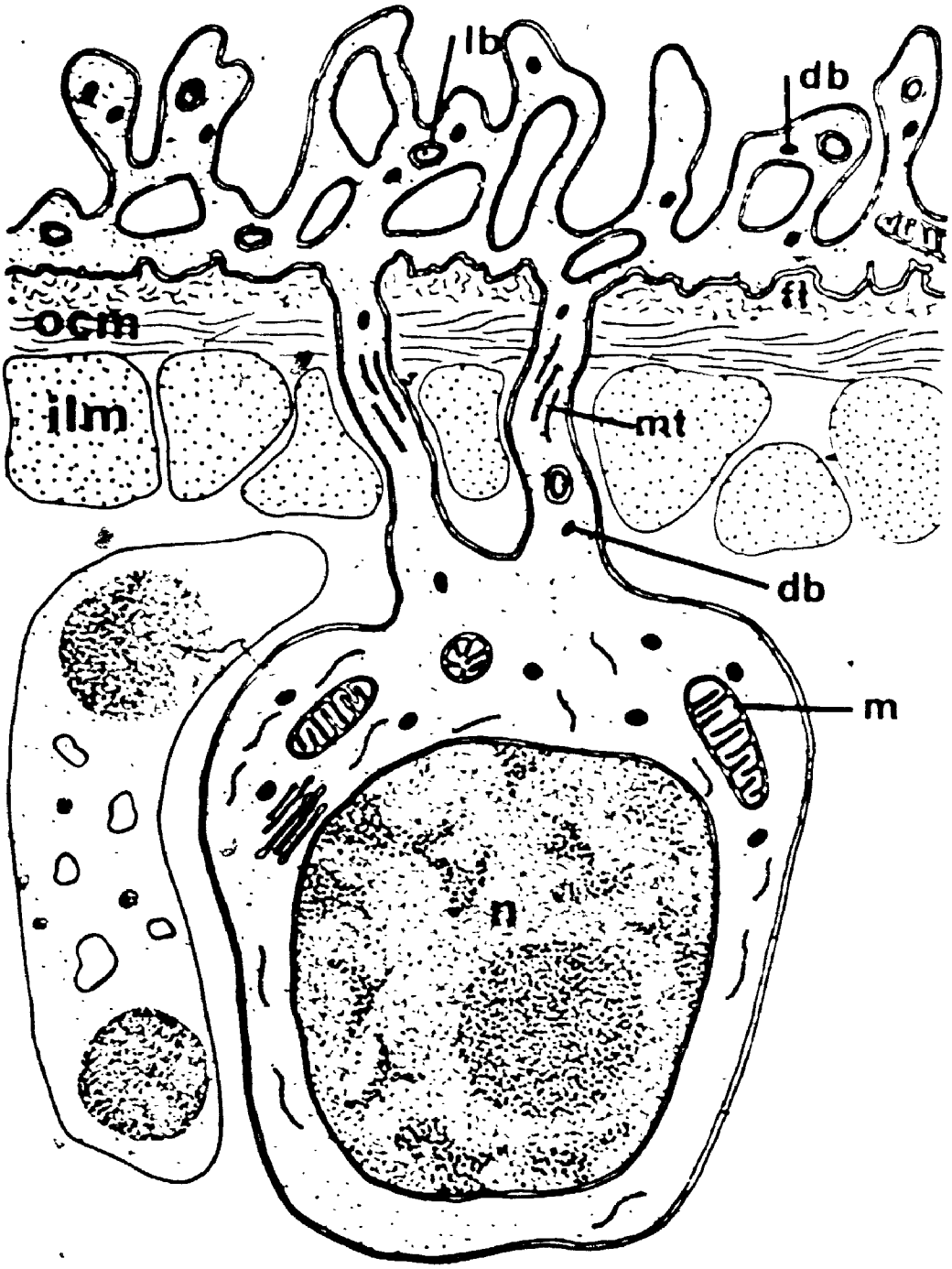

lb
db
dr
ocm
fl
ilm
mt
db
m
n

Figure 33. Electron micrograph of tegument of a schistosomulum from a hyperimmune duck (day 3) reacted with peroxidase-labelled rabbit anti-duck gamma globulin. Unstained except for peroxidase. X40000.
m - mitochondrion
per - peroxidase

Figure 34. Electron micrograph of tegument of day 3 initial infection reacted with peroxidase-labelled rabbit anti-duck gamma globulin. Unstained except for peroxidase. X60000.
ip - internuncial process
m - mitochondrion


pot

Figure 35. Schistosomula from an immune duck 3 days after exposure to cercariae and treated with benzpyrene. X280, uv light.
Note general fluorescence in tegument.

g - gut

Figure 36. Schistosomula from an initial infection 3 days after exposure to cercariae and treated with benzpyrene. Note lack of fluorescence in tegument and the obvious discrepancy in size between these worms and those in figure 35. X280, uv light.

g - gut

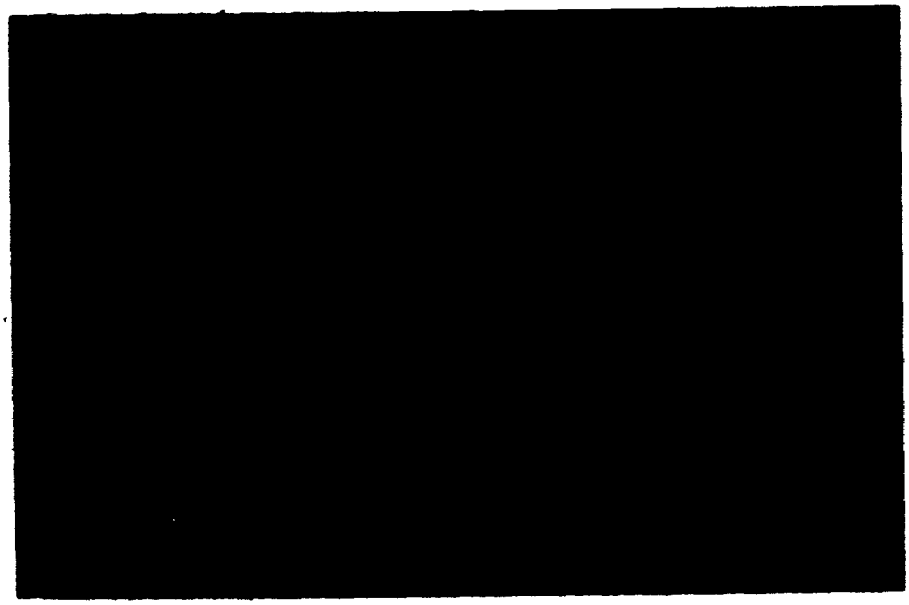


Figure 37. Egg-laying curves in four ducklings exposed to gamma radiation (Co^{60}). Ducks were exposed to cercariae 9 - 18 days after irradiation.

Figure 39. Distribution of T. ocellata in skin, lung, kidney, liver and intestine of ducks after initial exposure to cercariae (Data largely from Ellis, 1968).

Figure 38. Egg-shedding patterns of T. ocellata in groups of ducklings injected (i.p.) with massive amounts of normal serum, saline, or immune serum. Black areas on the figure indicate days when miracidia were recovered from a given bird. Hatched or dotted areas indicate days for which data are not available.

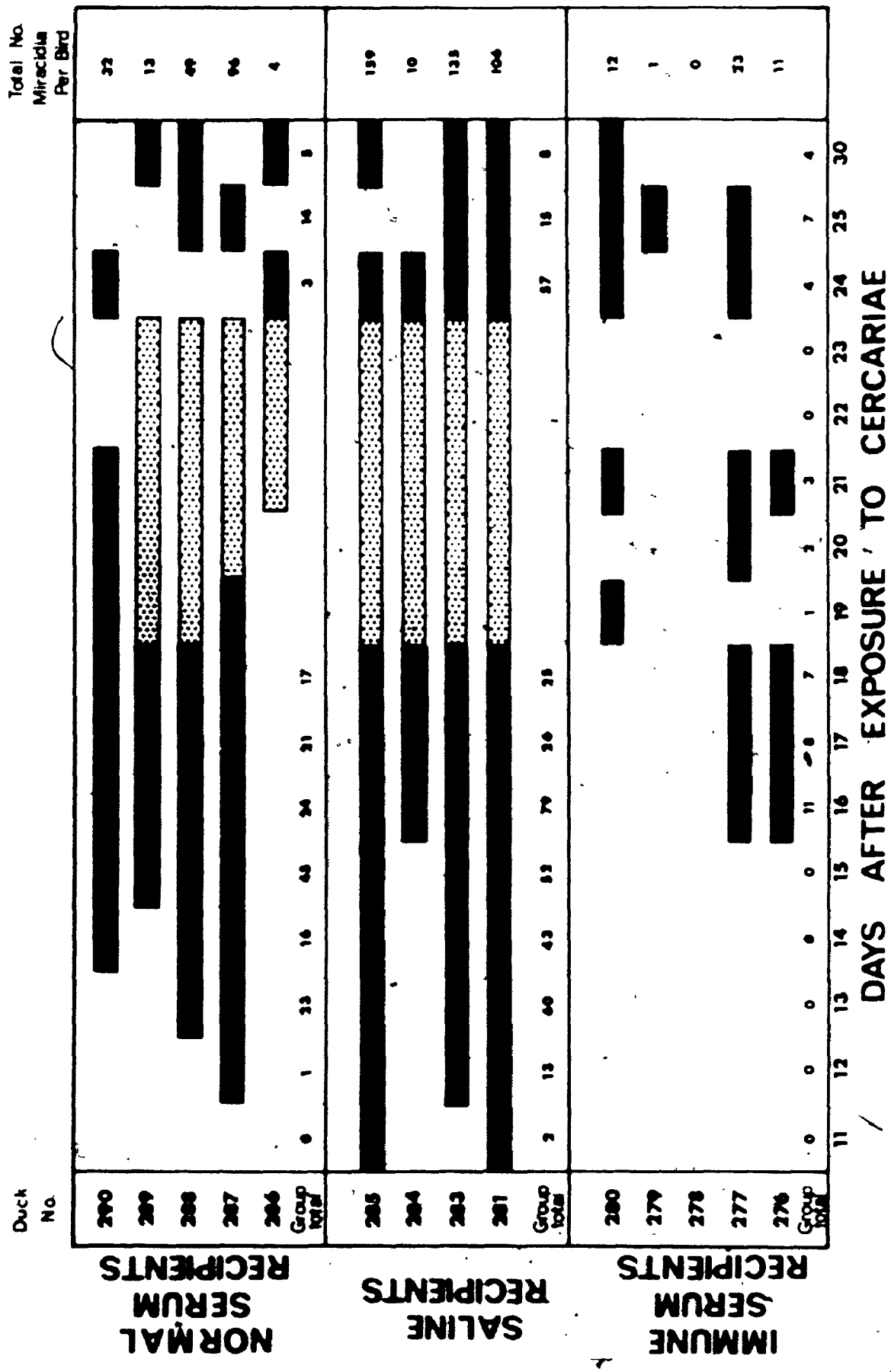

NORMAL SERUM RECIPIENTS
SALINE RECIPIENTS
IMMUNE SERUM RECIPIENTS
Duck No.
Total No. Miracidia Per Bird
DAYS AFTER EXPOSURE TO CERCARIAE

Figure 39. Distribution of T. ocellata in skin, lung, kidney, liver and intestine of ducks after initial exposure to cercariae (Data largely from Ellis, 1968).

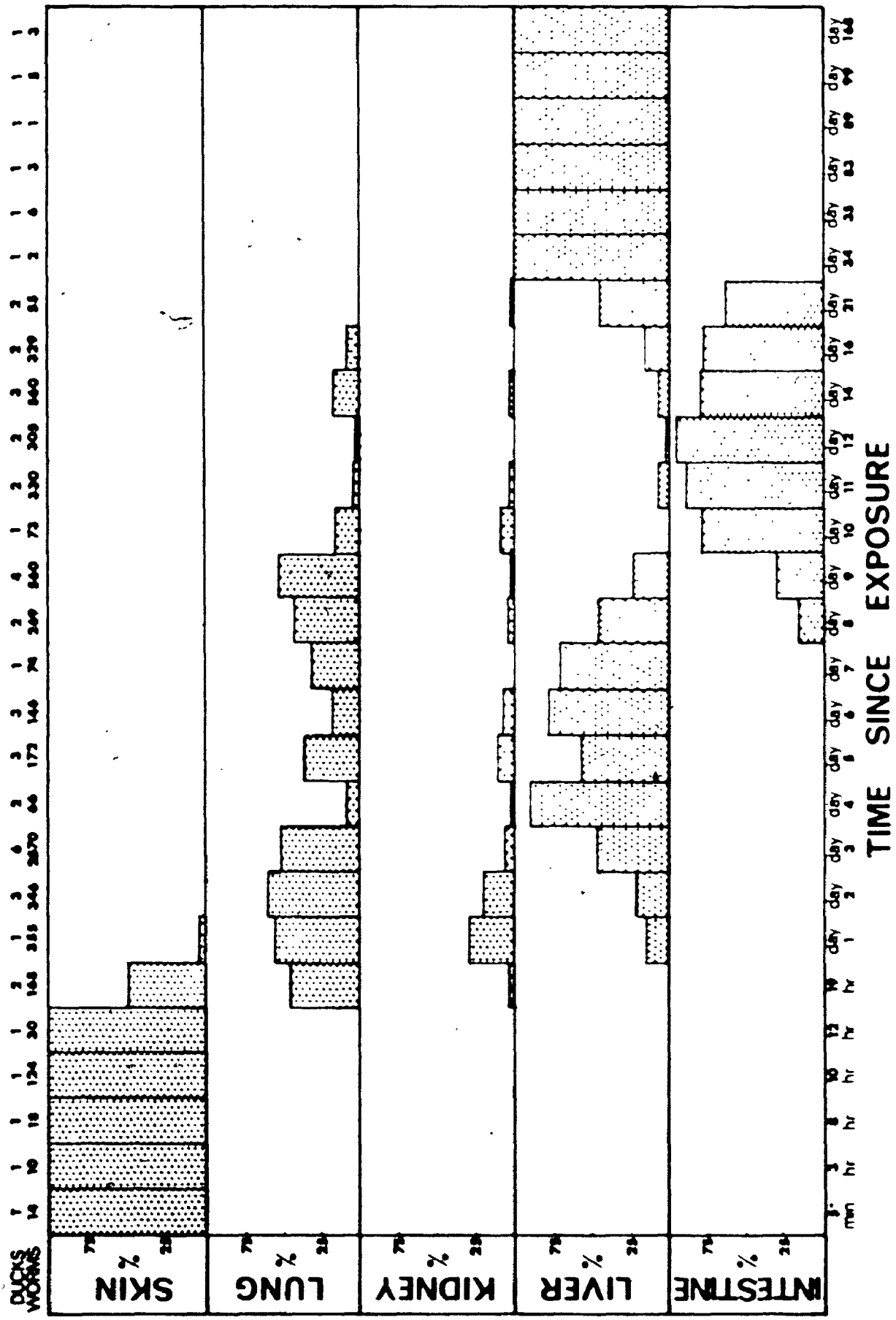

SKIN
LUNG
KIDNEY
LIVER
INTESTINE
%
TIME SINCE EXPOSURE

Figure 40. A comparison of the per cent schistosomula found in lymphoid areas of lung after initial and challenge infections with cercariae of T. ocellata.

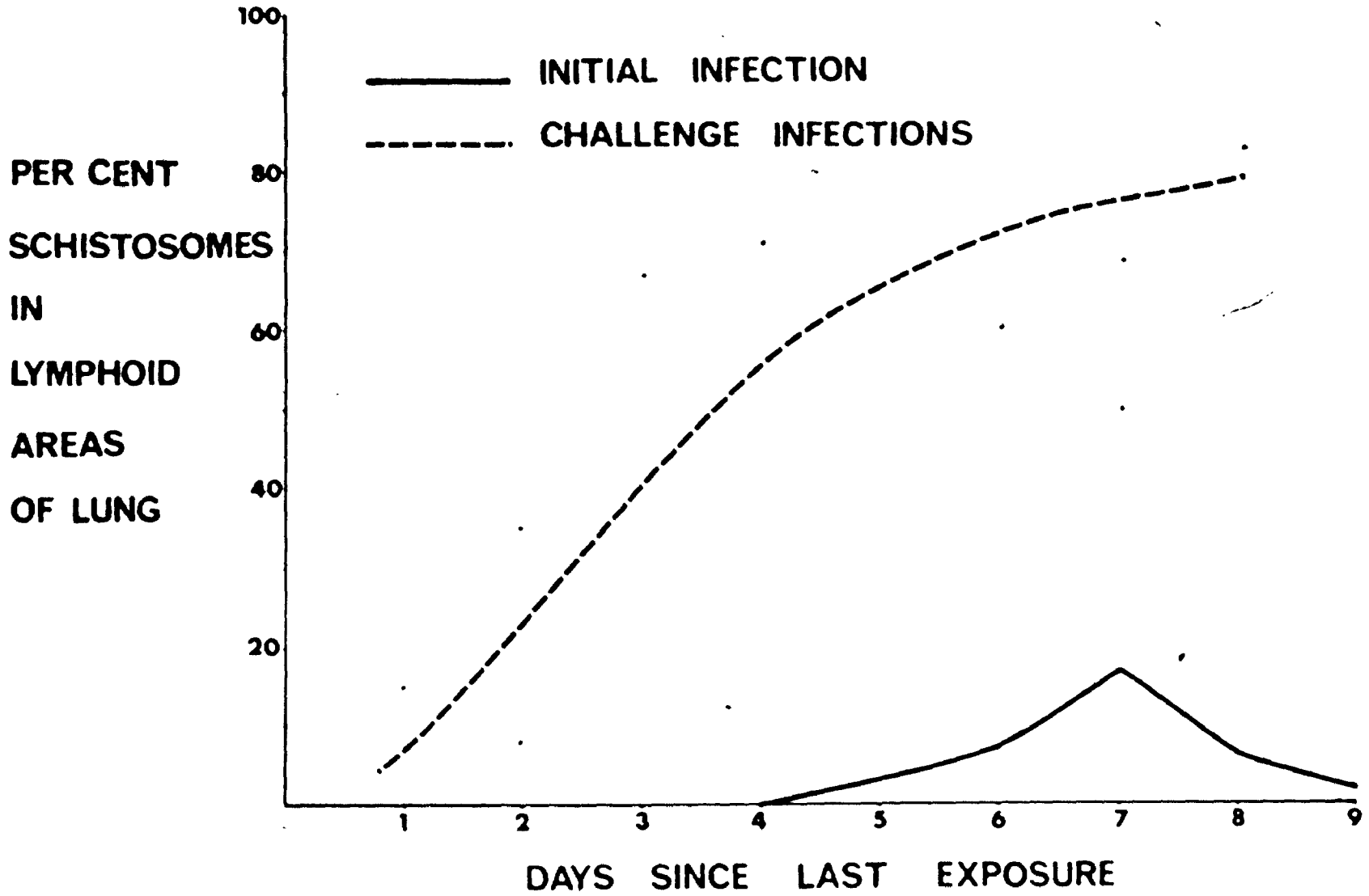
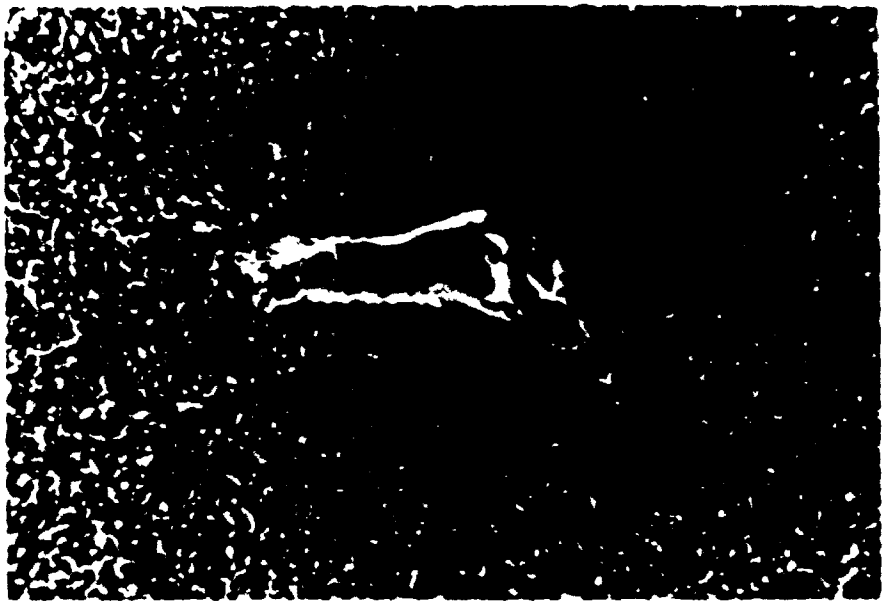

INITIAL INFECTION
CHALLENGE INFECTIONS
PER CENT SCHISTOSOMES IN LYMPHOID AREAS OF LUNG
100
80
60
40
20
1
2
3
4
5
6
7
8
9
DAYS SINCE LAST EXPOSURE

Figure 41. Section of liver 35 days after initial infection with *T. ocellata* showing worm in central vein with virtually no accompanying reaction. X280. s - adult *T. ocellata*



APPENDIX I

FIXATIVE

Davidson Fluid	-	200 ml. formalin
	-	300 ml. 95 % ethyl alcohol
	-	100 ml. glycerine
	-	100 ml. glacial acetic acid
	-	300 ml. distilled water

STAINS

Ehrlich's alum haematoxylin (Age 1 year)

	-	28 gm. haematoxylin
	-	120 ml. glacial acetic acid
	-	1200 ml. absolute alcohol
	-	1200 ml. distilled water
	-	1200 ml. glycerine
	-	400 gm. aluminium ammonium sulphate

Bowie's eosin	-	100 ml. 1 gm. alcoholic eosin in 80 % alcohol
	-	100 ml. 1 gm. eosin B in 30 % alcohol
	-	100 ml. 1 gm. eosin Y in distilled water

APPENDIX II

A COUPLING OF PEROXIDASE TO RABBIT GAMMAGLOBULIN

1. 30 mg. rabbit anti-duck gammaglobulin which had been made and purified for FA technique by Smith (1971) was added to 72 mg horseradish peroxidase (Sigma type II) in 0.1 M sodium phosphate buffer (pH 6.8) to a final volume of 6.0 ml.

2. 0.3 ml. of 0.1 % aqueous glutaraldehyde was added dropwise with gentle stirring and the solution allowed to stand for 2 h at room temperature (occasional stirring).

3. 3 mls saturated ammonium sulphate (0° C) was added and the solution cooled to 0° C for 10 minutes.

4. Solution was transferred to a centrifuge tube washing the solution in with (ml of 1/3 saturated ammonium sulphate.

5. Solution was centrifuged (cold) at 30000 g for 10 minutes.

6. Supernatant was removed and discarded.

7. Residue was resuspended in 3 mls 1/3 sat. ammonium sulphate and centrifuged for 10 minutes at 0° C (30,000 g).

8. Steps 6 and 7 were repeated.

9. Residue was resuspended in approximately 2 mls. phosphate buffer (pH 7.5).

10. This was dialyzed against PBS at 4° C (pH 7.5) until all traces of sulphate were gone ($BaCl_2$ test).

11. The dialysates were centrifuged (0° C) at 30,000 g for 10 minutes, the supernatants carefully removed, the pH adjusted

to 7.5 with K_2HPO_4 (1M) and 0.5 ml aliquots were pipetted into individual tubes and frozen.

B GRAHAM AND KARNOVSKY METHOD FOR PEROXIDASE

1. Worms were placed in a solution of 75 mg 3, 3'- diamino benzidine (free base) in 100 ml 0.05 M tris buffer (pH 7.6) for 1 h at 4° C.

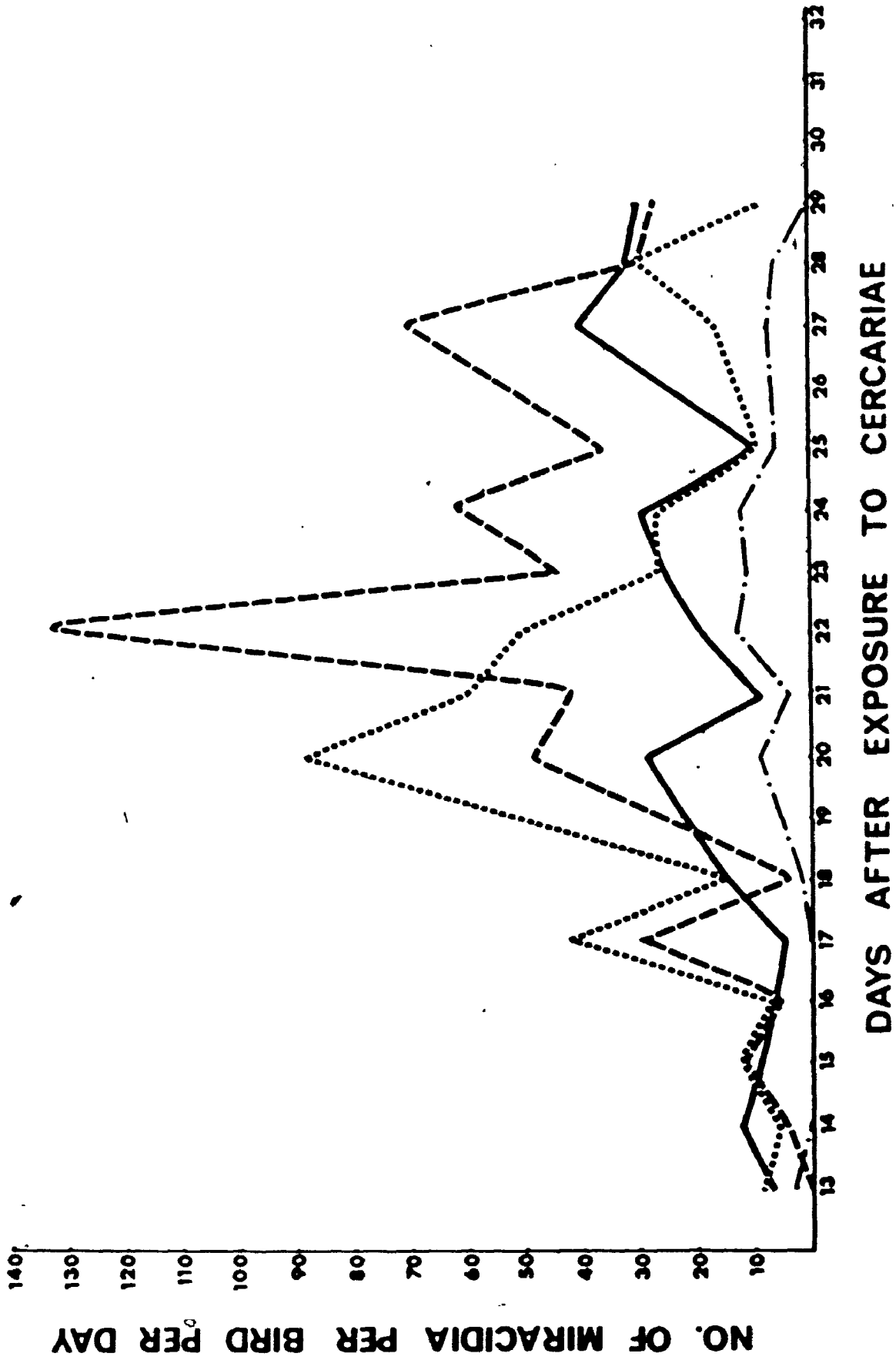
2. Incubated for 30 minutes at room temperature in a solution of 75 mg 3, 3'- diamino benzidine in 0.001 % peroxide in 100 ml. 0.05 M tris buffer (pH 7.6).

3. Washed in 3 changes distilled water 30 minutes each at room temperature.

4. Osmicated 1 h in 1 % O_s O_4 buffered in veronal.

5. Dehydrated and embedded in Epon.

6. Sectioned and examined in the EM without further staining.


NO. OF MIRACIDIA PER BIRD PER DAY
140
130
120
110
100
90
80
70
60
50
40
30
20
10
13
14
15
16
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DAYS AFTER EXPOSURE TO CERCARIAE

APPENDIX III

Percent of egglaying of *T. ocellata* in ducks occurring after the maximum 24 h period in normal and irradiated birds

A. NORMAL ADULT DUCKS

Duck No.	% after peak
1	75
2	46
3	70
4	76
5	41
6	74
7	69
8	54
9	52
10	66

B. NORMAL DUCKLINGS

Duck No.	% after peak
11	32
12	74
13	64
14	63
15	74
16	51

C. IRRADIATED DUCKLINGS

Duck No.	Radiation Dose (Rads)	% after peak
206	400	61
338	500	40
339	500	89
372	600	77
340	600	69
342	700	67
343	700	89

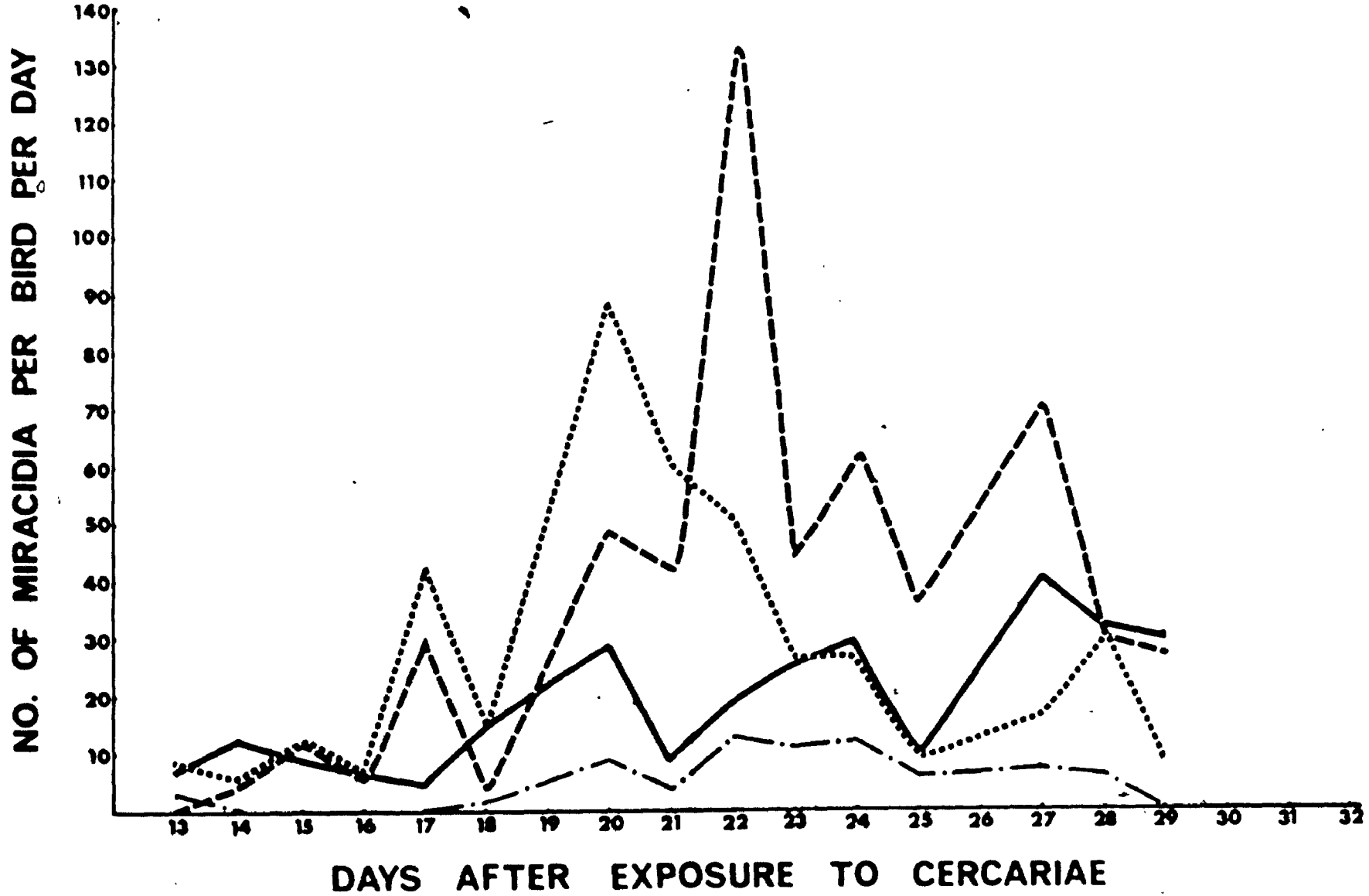
344	800	47
345	800	74

APPENDIX IV

To test the uniformity of infections after exposure to similar numbers of cercariae, 4 ducklings were secured with one foot at the perimeter of a circular dish, each individual being equally distant from each other, and a uniform but light suspension of cercariae was added. The whole apparatus was kept in the dark during the 90 minute exposure to avoid unequal cercarial distribution due to phototactic responses.

The resulting egg-laying curves (fig. 1) displayed erratic patterns confirming Rau's (1969) discovery that no correlation exists between the numbers of cercariae entering a duck and the numbers of parasite eggs produced.

Figure 1. Egg laying curves of T. ocellata in four individual ducklings exposed simultaneously to a uniform, light suspension of cercariae.


NO. OF MIRACIDIA PER BIRD PER DAY
140
130
120
110
100
90
80
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DAYS AFTER EXPOSURE TO CERCARIAE

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